STUDIES ON THE EFFECT OF ADDITIVES ON AMPHIPHILIC DRUGS SOLUTIONS

ABSTRACT

THESIS

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Interest in research on amphiphilic systems in solutions is increasing day-by-day. This is not only because of their wide variety of applications in industries, but also due to the development of new and more powerful experimental and theoretical tools for probing the microscopic behavior of these systems.

The name amphiphile is sometimes used synonymously with surfactant. The word is derived from the Greek word *amphi*, meaning both, and the term relates to the fact that all surfactant molecules consist of at least two parts, one which is soluble in a specific fluid (the lyophilic part) and one which is insoluble (the lyophobic part). When the fluid is water one usually talks about the hydrophilic and hydrophobic parts, respectively. The ambivalence of amphiphiles towards an aqueous environment leads to phenomena which solutions of simpler solute molecules and water do not exhibit.

Amphiphiles play an essential role in the existence of life and are widely used in the industry, medicine, pharmacology, etc. The single feature of amphiphiles that gives rise to such broad utility is their ability to coexist with and function as an interface between polar and non-polar phases. This ability is determined by a balance between ionic and dipolar interactions with polar media and dispersion interactions with non-polar media.

Solutes showing hydrophobic self-association may be classified into four categories on the basis of the chemical structure: (A) flexible chain compounds (surfactants, etc.), (B) aromatic or heterocyclic ring or fused ring structures (dyes,

drugs, etc.), (C) alicyclic fused compounds (bile salts, etc.), and (D) macromolecular solutes (proteins, etc.). The self–association behavior must relate to the chemical structure of the solutes. The simplest type of association, viz., dimerization, may take place in all the self-associating systems being considered. The formation of higher multimers may overshadow it, however, more or less completely.[1]

One of the most characteristic properties of amphiphiles is their capacity to aggregate in solutions. The narrow concentration range over which these aggregates form has been called the critical micelle concentration (cmc) and the aggregates that form are known as micelles.

Among the factors known to affect the cmc markedly in aqueous solutions are: (i) structure of the amphiphiles, (ii) presence of various additives in the solution, (iii) experimental conditions such as temperature, pH, pressure, solvent, etc.

When two neat liquids are brought together, they may either mix into a homogenous solution or they may form two solutions, where in each case one of the components can be regarded as the solvent. Normally in the latter case, the compositions of the two phases in equilibrium become more equal when the temperature is increased. There are, however, frequent exceptions from this expected behavior. Such systems are represented by liquids that are completely miscible at low temperatures but phase separate when heated. This is observed by

the clear solution present at low temperatures suddenly becoming cloudy on heating. The temperature where this occurs is referred to as the cloud point (CP).

The basis of the phase separation (or Cloud point) stems from the well-known phase phenomenon exhibited by some surfactant micellar solutions. Namely, upon appropriate alteration of the conditions (i.e., temperature or pressure change, addition of salt or other additive, etc.), the separation of an aqueous surfactant micellar solution into a concentrated phase containing most of the surfactant (termed surfactant-rich, micellar, or coacervate phase) and a dilute aqueous phase containing low concentration of surfactant is observed. Any component(s) originally present that binds to the micellar aggregate in solution can thus be extracted from the original solution and concentrated in the small volume element of the surfactant rich phase.[2]

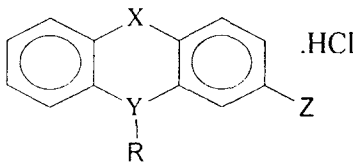
Generally, the clouding behavior would not happen in ionic surfactant systems because of significant electrostatic repulsions between the charged aggregates. Nevertheless, previous researches showed that aqueous solutions of some ionic surfactants with high salt concentration,[3,4] salt free aqueous solutions of certain ionic surfactants with large head groups or large counterions [5] and some mixed cationic and anionic surfactant solutions also exhibited the above behavior. The mechanism of the behavior in these ionic surfactant solutions is still an open question.[6]

Recently, Kim and Shah[7,8] have observed the CP phenomenon in amphiphilic drug amitriptyline hydrochloride solutions and have explored the effect of additives. Also, Kabir-ud-Din and his group[9,10] have studied CP phenomenon in some amphiphilic drug solutions and examined the effects of different additives.

When using drugs it should be kept in mind that normal human body temperature is typically 12 degrees above ambient. Even if the CP of pure drug in buffer is above this temperature, it may decrease in presence of additives, especially with surfactants (which are used as drug carriers). At CP the drug concentrates into a small volume, leading to localized high concentration at particular site. This may lead to aggregation causing a change in biological activity. With this idea in mind, effect of various additives, viz., electrolytes, non-electrolytes, alcohols, amino acids, sugars, surfactants and their concentration effects have been examined in CP and dye solubilization of drugs.

In the general introduction (**Chapter I**), a detailed account of the behavior of amphiphile systems, the factors responsible for the formation of various aggregation patterns, their solution properties, e.g., cloud point phenomenon, etc. is described. From the literature survey it appears that exploring CP in amphiphilic drugs in presence of additives is a recent phenomenon. Experimental details are provided in **Chapter II**.

Chapter III contains cmc data for antidepressant (NOT and CLP) and phenothiazine (PMZ) drugs (Scheme 1) in aqueous media. Effects of some selected additives on the cmc of these drugs in aqueous solutions are also reported. The cmc values of drugs decrease with the addition of surfactants and NaCl. The results of conductivity measurements on drug-surfactant systems indicate mixed micellization. Negative values of interaction parameter (β) and excess free energy of micellization (ΔG_{ex}) suggest attractive interactions in the mixed micelles. Mole fractions of surfactants in micelles are higher than expected for ideal mixing. Activity coefficients, f_1 and f_2 values also show non-ideal mixing. Adsorption of Cl^- to the drug molecules results in cmc decrease.



Nortriptyline, NOT: X = CH_2CH_2; Y= C; R= >CH-$(CH_2)_2$-NH (CH_3); Z = H

Clomipramine, CLP: X = CH_2CH_2; Y = N; R = -$CH_2(CH_2)_2$-N$(CH_3)_2$; Z = Cl

Promazine, PMZ: X = S; Y = N; R = $(CH_2)_3$-N $(CH_3)_2$; Z = H

Scheme-1

Studies on the effect of various additives on the CP values and dye solubilization of two antidepressant (NOT, CLP) and phenothiazine (PMZ) drugs

are described in **Chapter IV**. The CP's of 50 mM drug (for NOT, 30 mM) solutions (prepared in 10 mM sodium phosphate buffer) were found to decrease with increasing pH, both in the absence as well as in the presence of additives. Increase in pH leads to deprotonation of nitrogen atom of tertiary amine portion of the drug molecule. This effect reduces the micellar surface charge and electrostatic repulsion among monomers which, in turn, increases the aggregation number and compactness of micelles. Consequently, clouding takes place at lower temperatures. The dye solubilization was found to increase with increasing pH, which indicates that the size of the micelles is increasing. The resulting micelles dissolve more dye and hence the absorbance increases with increasing pH.

Addition of increasing amounts of salts (NaF, NaCl, NaBr, LiBr, KBr) to 50 mM drug (for NOT 30 mM) solutions caused continuous increase in CP. On the basis of these studies the binding-effect orders of counterions and co-ions have been deduced, respectively, as: $Br^- > Cl^- > F^-$ and $Li^+ < Na^+ < K^+$). The presence of counterions (F^-, Cl^- and Br^-) decreases the surface area occupied per drug head group (a_o) leaving l_c and v (length and volume of the drug monomer respectively) unaffected. Hence, packing parameter, R_p ($= v/a_o l_c$), increases and micelles grow. However, as these ions are hydrated their closer approach to micelles increases the micelle hydration and CP increases. Micellar growth in presence of counterions is confirmed by an increase in visible intensity of Sudan III in drug-counterion systems. Similar trend are observed with co-ions also. In this series, Li^+ is highly

hydrated (crystal radius: 0.60Å, hydrated radius: 3.28Å) while K^+ is least hydrated (crystal radius: 1.33Å, hydrated radius: 3.31Å). Thus, Li^+ decreases the availability of water to the micelles which results in a slow increase in CP compared to the increase with K^+ or Na^+ (the extent of decrease of availability of water to the micellar head group region, as per their crystal radii, is $K^+ > Na^+ > Li^+$).

The similar trend of increasing CP and dye solubilization with addition of increasing amount of quaternary bromides (TMeAB, TEtAB, TPrAB, TBuAB, TPeAB) to 50 mM drug (for NOT 30 mM) was found to be dependent upon the alkyl chain length of the particular salt. The quaternary ammonium ions (QA^+) are water structure formers and this effect increases with increase in the length of alkyl group. The CP rising in the case of QA^+ cations is ascribed to adsorption/mixed micelle formation predominating over water structure formation. In such case, micelles would experience greater intermicellar repulsion and consequently have increase in CP's and absorbance.

Urea and alkylureas were found to decrease the CP, which were found to be dependent upon the number of methyl groups present in the urea molecules. Contrary to this, thioureas increased the CP slightly. However, the presence of methyl groups has similar effect as in alkylureas. Urea replaces water from the interfacial region: therefore, CP decreases with urea addition. Rate of decrease in CP increases as the number of methyl group increases in urea. Inclusion of

mono-, -di or tetramethyl groups in urea increases the size of alkyl ureas and therefore, more water is replaced by their addition. The opposite effect of thiourea may be due to the difference in nature of >C= S and >C= O bonds: the >C= O is stronger than >C= S (as O is more electronegative). Therefore, electrons around S atom will be delocalized, thus making the S-atom electron deficient, i.e., a Lewis acid. As a result, the S-atom in thiourea would behave like a positive center, and repulsion between thiourea and drug micelle could be responsible for the observed CP increase.

All the sugars decrease the CP of all the three drugs. Sugars are known as water structure makers and enhance the association of water molecules by hydrogen bonds. Thus sugars decrease the availability of water to the head groups and lower CP.

The adsorption of non-electrolytes such as urea and sugars at the micelle-water interface originates a restriction of micellization and is responsible for a decrease in the visible absorbance.

The effect of amino acids on the CP of drug solutions depends upon their acidic/basic as well as polar/non-polar characteristics. The negatively charged side chain of acidic amino acids would interact with tertiary amine of the drug. This will allow further hydration of the micelles, hence the CP of the system increases. Hydrophobic non-polar and uncharged polar amino acids, on the contrary, would partition in micellar interior or bulk water, respectively. In either case the

hydration of micelles is not affected and the CP as well. The basic amino acids, being polar, partition in the head group region with the result that certain amount of water near the head group region is replaced; the observance of CP phenomenon at a lower temperature. Hydrochloride salts bear a positive charge on them and their interaction with the drug micelles would result in increased micelle–micelle repulsion. The CP would then increase which indeed is the case.

Short chain alcohols increase the CP slightly but higher ones with long chain (due to their hydrophobic nature) decrease it. Short chain alcohols are hydrophilic in nature and due to favorable interactions with water modify the water structure, making it more compatible with the single surfactant molecules. Long chain alcohols are only partially soluble in water and, being increasingly solubilized by the micelles, they are expected to affect the micelle size. They solubilize in the micelles with their hydroxyl groups toward the surface. Therefore, larger aggregates would form and CP decreases.

With ethanediol and propane-1.2–diol. CP remains almost constant. Diols, being hydrophilic and highly miscible in water (as they contain two-OH groups on hydrophilic ethane or propane molecules), remain in aqueous phase at all concentrations and would not affect the micelle hydration. However, cycloalkanols show different behavior; with allylalcohol and cyclopentanol, unlike cyclohexanol, the CP decrease is not pronounced in the beginning. The results are

in conformity to the relative solubility of the cycloalkanols and prove cyclohexanol to be the highest on hydrophobicity scale in the present system.

Surfactants are used extensively in drug delivery as drug carriers. At fixed [drug] (50 mM, in case of NOT 30 mM) surfactants are found to affect the CP in accordance to their nature and structure: cationic (conventional as well as gemini) and non-ionic surfactants show continuous increase, whereas anionic surfactants show an increase followed by a decrease.

The added cationic surfactants exist in the drug solutions as monomers, micelles and mixed micelles depending on the following characteristics of the cationic surfactants used: (a) length of the hydrophobic tail, (b) nature of the head group, (c) nature of the counterion, (d) gemini *vs.* conventional, and (e) gemini spacer length. The degree of the increase in the CP depends on these characteristics.

The visible spectra of Sudan III show an increase in absorbance for drug-surfactant systems in comparison to that for pure drugs solutions. These surfactants form mixed micelles with drugs causing an increase in micellar size and dye solubility increases.

Non-ionic surfactants contain oxyethylene chains and hence are hydrophilic in nature; more heating is required to dehydrate these mixed micelles. Hence CP is increased.

At low concentrations, the anionic surfactant molecules adsorb at the micellar interface and inhibit micellization resulting in an increase in CP. At higher concentrations, the added surfactant increases the compactness of the micelles and leads to a decrease in CP.

At any fixed concentration of additives, a decrease in CP is observed with increased pH due to deprotonation of drug micelles/monomers. This causes lowering of the electrostatic repulsion (among micelles) with a concomitant larger decrease in CP.

Increase in drug concentration in presence of fixed amounts of additives increase the CP. The number, size, and charge of micelles increase with the increase in the drug concentration, which increases both inter-micellar and intra–micellar repulsions, causing increase in the CP values.

In the case of different fixed drug concentrations (without additive), the absorbance increase with increasing drug concentration indicates increase in the dye solubility. Obviously, the number and size of micelles increase with the increase in the drug concentration and hence the dye solubility.

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Certificate

This is to certify that the thesis entitled **"STUDIES ON THE EFFECT OF ADDITIVES ON AMPHIPHILIC DRUGS SOLUTIONS"** is the original work carried out by **Mr. Mohammed Dabi Ali Al-Ahmadi** under my supervision and is suitable for submission for the award of **Ph.D.** degree in **Chemistry**.

(Dr. Mohd. Akram)

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(Mohammed Dabi Ali Al-Ahmadi)

List of Publications

1. Study of the Additive Effect on the Cloud Point of Nortriptyline Hydrochloride Solutions.
   Kabir-ud-Din, **Mohammed D. A. Al-Ahmadi**, Andleeb Z. Naqvi, Mohd. Akram
   J. Surf. Detergents, **10**, 231–236 (2007).

2. Study of the Cloud Point of Nortriptyline Hydrochloride: Effect of Additives.
   Kabir-ud-Din, **Mohammed D. A. Al-Ahmadi**, Andleeb Z. Naqvi, Mohd. Akram
   Chem. Eng. Comm., **195**, ... (2008) (in press).

3. Effect of Electrolytes on the Cloud Point and Dye Solubilization of Promazine Hydrochloride Solutions.
   Kabir-ud-Din, Mohmmed **D. A. Al-Ahmadi,** Andleeb Z. Naqvi, Mohd. Akram
   J. Dispersion Sci. Technol. (in press).

4. Phase Separation Study of Surface-Active Drug Promazine Hydrochloride in Absence and Presence of Organic Additives.
   Kabir-ud-Din, **Mohammed D. A. Al-Ahmadi**, Andleeb Z. Naqvi, Mohd. Akram
   ISSCS-2007, (Kolkata), Special Edited Volume (Eds. B. K. Paul and S. P. Moulik), World Scientific Publishers Pvt. Ltd., Singapore (in press).

5. Phase Separation Study of a Surface-Active Drug, Promazine Hydrochloride, in Presence of Surfactants and Ureas.
   Kabir-ud-Din, **Mohammed D. A. Al-Ahmadi**, Andleeb Z. Naqvi, Mohd. Akram
   Tenside, Surfactants, Detergents, **45**, ----- (2008) (in press).

List of Papers accepted / presented at Conferences

1. Effect of Urea and Alcohols on the Cloud Point of Nortriptyline Hydrochloride.
   Kabir-ud-Din, **Mohammed D. A. Al-Ahmadi**, Andleeb Z. Naqvi and Mohd. Akram
   9th CRSI National Symposium in Chemistry, Delhi University, Delhi, Feb. 1– 4, 2007.

2. Effect of Electrolytes on Cloud Point of Promazine Hydrochloride.
   Mohd. Akram, **Mohammed D. A. Al-Ahmadi,** Andleeb Z. Naqvi and Kabir- ud-Din
   13th National Conference on Surfactants, Emulsions and Biocolloids with Special Focus on Biomimetic Systems, BITS, Pilani, Feb. 22–24, 2007.

3. Clouding Phenomenon in Amphiphilic Drug Solutions: Role of Salts.
   Kabir-ud-Din, **Mohammed D. A. Al-Ahmadi**, Andleeb Z. Naqvi, Mohd. Akram
   2nd International Conference on Advances in Petrochemicals and Polymers, Bangkok, Thailand, June 25–28, 2007.

4. Phase Separation Study of Surface-Active Drug Promazine Hydrochloride in Absence and Presence of Organic Additives.
   Kabir-ud-Din, **Mohammed D.A. Al-Ahmadi,** Andleeb Z. Naqvi, Mohd. Akram
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Contents

Chapter–I

General Introduction

The name amphiphile is sometimes used synonymously with surfactant. The word is derived from the Greek word *amphi*, meaning both, and the term relates to the fact that all surfactant molecules consist of at least two parts, one which is soluble in a specific fluid (the lyophilic part) and one which is insoluble (the lyophobic part). When the fluid is water, one usually talks about the hydrophilic and hydrophobic parts, respectively. The ambivalence of amphiphiles towards an aqueous environment leads to phenomena which solutions of simpler solute molecules and water do not exhibit.[1]

Amphiphiles play an essential role in the existence of life and are widely used in the industry, medicine, pharmacology, etc.[2,3] The single feature of amphiphiles that gives rise to such broad utility is their ability to coexist with and function as an interface between polar and non-polar phases. This ability is determined by a balance between ionic and dipolar interactions with polar media and dispersion interactions with non-polar media.

When amphiphiles are dispersed in water, many types of aggregates can be formed, the solvated hydrophilic groups being located at the surface of aggregate.[1,4] The self-association gives rise to a rich variety of phase structures (Fig.1.1). The state of aggregation of amphiphiles in an aqueous solution is a complex function of their structure, the charge of molecules, and the aqueous solvent properties (concentration of the amphiphile, ionic strength, pH, temperature, etc.). Aggregation is not, however, just limited to aqueous solutions; it is sometimes observed in non-aqueous polar solvents such as ethylene glycol and non-polar solvents such as hexane (in the latter case giving

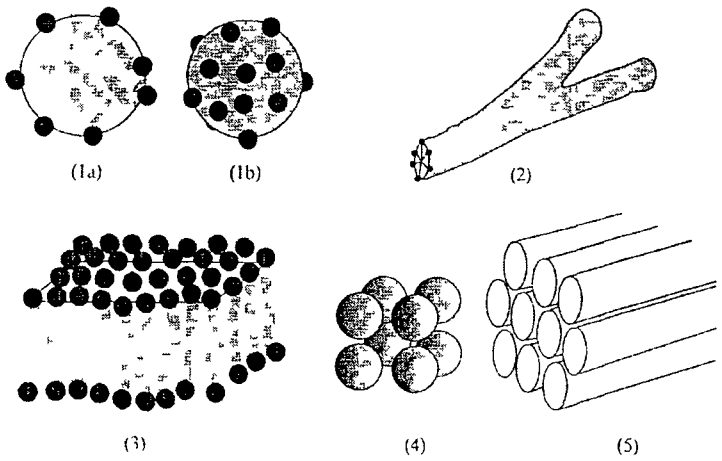


Fig. 1.1: *Examples of some of the (self-assembled) phase structures that can occur above the cmc with increasing concentration of amphiphile (1) spherical micelle ('a' as cross-section), (2) 'worm-like' micelle, (3) lamellar phase, (4) cubic phase, (5) hexagonal phase*

rise to inverse structures).[5]

Surfactants with aliphatic chains generally aggregate to micelles with aggregation numbers between 40 and 200, depending on their particular molecular structure.[6]

Solutes showing hydrophobic self-association may be classified into four categories on the basis of the chemical structure: (A) flexible chain compounds (surfactants, etc.), (B) aromatic or heterocyclic ring or fused ring structures (dyes, drugs, etc.), (C) alicyclic fused compounds (bile salts, etc.), and (D) macromolecular solutes (proteins, etc.).[7] The self-association behavior must relate to the chemical structure of the solutes. The simplest type of association, viz., dimerization, may take place in all the self-associating systems being considered. The formation of higher multimers may overshadow it, however, more or less completely.[7]

Critical Micelle Concentration

One of the most characteristic properties of amphiphilic molecules is their capacity to aggregate in solutions. Almost from the beginning of the study of the properties of amphiphilic solutions, it was recognized that their bulk properties were unusual and indicated the presence of aggregates in the solution. The concentration at which this aggregate formation occurs is usually fairly sharply defined and it can be identified by observing the behavior of any one of a number of equilibrium or transport properties of the solution. The narrow concentration range over which amphiphilic solutions show an abrupt change in physicochemical properties is called the critical micelle

concentration (cmc)[8-10] and the molecular aggregates that form above the cmc are known as micelles.

The micellar aggregation can be demonstrated by measuring solution properties, such as surface tension,[11-13] dye solubilization,[14,15] ^{1}H-NMR,[16-18] light scattering,[19-22] fluorimetry,[23,24] osmotic pressure,[20,25] electrical conductivity,[26-28] ultrasound velocity,[22] against amphiphile concentration. The cmc depends on the solution properties employed in the determination and therefore, differs with the method used. For this reason, the measured cmc values define a narrow concentration range.

Factors affecting the value of critical micelle concentration

Among the factors known to affect the cmc markedly in aqueous solutions are: (i) structure of the amphiphiles, (ii) presence of various additives in the solution, (iii) experimental conditions such as temperature, pH, pressure, solvent, etc.

(i) Structure of the amphiphiles

In aqueous medium, ionic amphiphiles have much higher cmc's than nonionic amphiphiles containing equivalent groups. Zwitterionic amphiphiles appear to have slightly smaller cmc's as ionics with the same number of carbon atoms in hydrophobic group. The position of hydrophobic group in hydrocarbon chain also affects the cmc. The closer the hydrophilic group to the center of the chain, the higher the cmc; due to the two branches of the chain partially shielding one another. In aqueous medium, the cmc's of ionic

amphiphiles decrease as the hydrated radius of the counterion decreases. The presence of double bond in the chain also causes an increase in cmc.

(ii) Presence of various additives in the solution

(a) Effect of electrolytes

Adding an indifferent electrolyte to an amphiphile/water system has a pronounced effect on the cmc. The forces of electrostatic repulsion between head groups of an ionic micelle are considerably reduced, enabling micelles to form more easily, that is, at lower concentration.[29] The effect being more pronounced for anionic and cationic than for zwitterionic surfactants, and more pronounced for zwitterionics than for non-ionics. The effect of concentration of electrolyte is given by the following equation[29]

$$\log \text{cmc} = -a \log C_1 + b \qquad (1.1)$$

where a and b are constants for a given ionic head at a particular temperature and C_1 is the total (monovalent) counterion concentration, in mole per dm^3. In case of non-ionics and zwitterionics, the effect is given by the equation [30]

$$\log \text{cmc} = -KC_s + \text{constant} \quad (C_s<1) \qquad (1.2)$$

where K is a constant for a particular surfactant, electrolyte, and temperature and C_s is the concentration of electrolyte in mole per dm^3.

The change in the cmc of non-ionics and zwitterionics on the addition of electrolyte has been attributed[31,32] mainly to the "salting-out" or "salting-in" (i.e., the effects of ionic size and decrease in dielectric constants) of the hydrophobic groups in the aqueous solvent by the electrolyte, rather than to the

effect of the latter on the hydrophilic groups of the amphiphile. Electrolytes capable of "salting-out" reduce the cmc of non-ionic surfactants while "salting-in" electrolytes increase the cmc. The effect of the anion and cation in the electrolyte are additive and appear to depend on the radius of the hydrated ion, that is the lyotropic number; the smaller the radius of the hydrated ion, the greater the effect. Thus the order of effectiveness in decreasing the cmc[30,33] is $1/2SO_4^{2-}$> F^- >BrO_3^- > Cl^- > Br^- > NO_3^-> I^-> CNS^- and NH_4^+> K^+> Na^+>Li^+ >$1/2Ca^{2+}$ Tetraalkylammonium cations appear to increase the cmc, the order of effectiveness being[30] $(C_5H_{11})_4N^+$ > $(C_4H_9)_4N^+$ > $(C_3H_7)_4N^+$ > $(C_2H_5)_4N^+$ > $(CH_3)_4N^+$

(b) Effect of organic additives

Small amounts of organic materials can have a significant influence on the cmc, and the properties of micellar solutions. A knowledge of the effects of organic materials on the cmc of amphiphiles is therefore of great importance for both theoretical and practical purposes. The organic additives affect the cmc by being incorporated into the micelle or by modifying solvent-micelle or solvent-amphiphile interactions

An important aspect of the behavior of the micelles is that they are able to act as sites for the dissolution of lipophilic (i e., fat soluble) molecules. It is common practice to divide organic materials into two groups, depending on their mode of action in influencing the cmc. Group A is composed of molecules (like alcohols with moderate to long hydrocarbon chains) that appear to be adsorbed in the outer regions of the micelles, forming a palisade (i.e , fence-

like) structure with the amphiphile molecules. This lowers the free energy of micellization to more negative values and so reduces the cmc; such molecules can also influence the micelle shape. Straight chain molecules have the most marked effect, the latter reaching a maximum when the length of the hydrophobic chain of the additive is about the same as that of the amphiphile. A decreased electrostatic repulsion between ionized head groups, and reduction in steric hindrance for non-ionic amphiphiles have been proposed as likely explanations for these observed effects.

Group B materials alter the cmc at substantially higher bulk concentrations and probably exert their influence through modification of the bulk water structure. The effect is usually discussed in terms of whether the additive is a (water) *structure maker* or a *structure breaker*. Typical "structure makers" are xylose and fructose[34] and "structure breakers" are urea and formamide.[35]

Structure breakers increase the cmc of amphiphiles in aqueous solutions, exerting their strongest influence on non-ionic amphiphiles of the polyethylene oxide type. Presumably the presence of a structure breaker reduces the amount of water structure that the hydrophobic residues of the amphiphile can induce. The entropy increase on micelle formation is thus reduced and so the cmc is raised. The concept is not, however, a very straightforward one to apply. Even where the solute is able to interact very strongly with water its effect may be overall structure breaking, firstly because it has to pull water from its existing

structure and secondly, because the resulting entity may substantially disrupt the remaining water structure.[36]

(iii) Effect of experimental conditions

(a) Temperature

The effect of temperature on the cmc of amphiphiles in aqueous medium is complex. Temperature increase causes decreased hydration of the hydrophobic group, which favors micellization. However, temperature increase also causes disruption of the structured water surrounding the hydrophobic group, an effect that disfavors micellization. The relative magnitude of these two opposing effects, therefore, determines whether the cmc increases or decreases over a particular temperature range. From the data available, the minimum in the cmc-temperature curve appears to be around 25 °C for ionics[37] and around 50 °C for non-ionics.[38, 39] Data on the effect of temperature on zwitterionics are limited. They appear to indicate a steady decrease in the cmc of alkylbetaines with increase in the temperature in the range 6-60 °C.[40, 41]

Effect of temperature on non-ionic surfactants is straightforward. The cmc of non-ionic surfactants based on polyethylene oxide decreases with increasing temperature as the hydrophobicity of the PEO chain decreases.[42] Several factors contribute to the decrease in hydrophilicity at a higher temperature but the three most important are:

(i) change in water structure around the EO groups,

(ii) change in hydrogen bonding to the EO groups, and

(iii) change in preferred conformations of EO chain.

(b) pH

Where amphiphile molecules contain ionizable groups such as $-NH_2$, $-(CH_3)_2N\rightarrow O$ and –COOH, the degree of dissociation of the polar group will be dependent on pH.[43] In general, the cmc will be high at pH values where the group is charged (low pH for $-NH_2$ and $-(CH_3)_2N\rightarrow O$, high pH for –COOH) and low when uncharged. Some zwitterionic surfactants become cationic at low pH, a change that can be accompanied by a rapid rise in the cmc,[44] or a more modest rise[45] depending on the structure and hence hydrophilicity of the zwitterionic form.

(c) Pressure

Many reports have appeared on the effect of pressure on micelle formation of ionic[46-51] and non-ionic amphiphiles.[52] With pressure cmc of ionic surfactants increases up to 1000 atm followed by a decrease above this pressure.[53-58] Such behavior has been rationalized in terms of solidification of the micellar interior, increased dielectric constant of water,[54] and other aspects related to water structure.[55] For non-ionic amphiphiles, the cmc value increases monotonously and then levels off with increasing pressure. La Mesa[56] has also discussed the effect of pressure on the cmc.

(d) Solvent medium

In ethylene glycol, the cmc of surfactants decreases as the length of the hydrophobic chain increases, but the change is much smaller than that in

water.[59] For polyoxyethylenated non-ionic solutions in benzene and carbon tetrachloride, cmc's decrease with increase in the length of the polyoxyethylene group at constant hydrophobic chain length.

The cmc's in benzene for alkylammonium carboxylates increase with increase in the length of the alkyl chain of the anion but decrease with increase in the length of the alkyl chain of the cation; in carbon tetrachloride, there is no significant change in the value of the cmc with these structural changes. The cmc is lower in D_2O than H_2O for different amphiphiles.[60,61] The hydrophobic bonds are expected to be stronger in D_2O than H_2O.[62] Micelles in D_2O are larger than H_2O.[63]

Molecular Shape

The degree of interaction between water and amphiphilic molecules can be expressed by the molecular shape. The molecular shape is a term for how large the lipophilic region is compared to the hydrophilic region of the molecule and thus not depends on the actual atoms and covalent bondings within the molecule. Amphiphiles, which form spherical micelles in water, have a conical shape in this aggregate type. Cylindrically formed molecules have a polar region that is equal to the non-polar, whereas wedge-shaped molecules have a large non-polar region thus forming, for example, reverse micelles. Substances with one hydrocarbon chain often belong to the conical group whereas substances with two chains or one chain with unsaturations, giving kinks, belong to cylinders and wedges.

The shape of the amphiphile aggregate is mainly determined by amphiphile packing parameter which is a dimensionless group relating the volume of the hydrocarbon tail of the amphiphile molecule (v), the length of the hydrophobic chain (l_c), and the head group area (a_o) [64,65] (Fig 1.2). The packing parameter (R_p) is given by the expression

$$R_p = v/a_o l_c \tag{1.3}$$

The optimum cross sectional area per amphiphile molecule is observed experimentally by X-ray diffraction of bilayer systems, while the volume and length of hydrocarbon tail may be calculated following Tanford [66]

$$v = (27.4+26.9n)\ \text{Å}^3 \tag{1.4}$$

$$l_c = (1.5+1.265n)\ \text{Å} \tag{1.5}$$

(n is the number of carbon atoms in the amphiphile's hydrocarbon chain).

Considering the geometric dimensions, the volume, and the surface area of each association structure yield critical conditions for the formation of each of the following shapes:

Values of $R_p = v/a_o l_c$	Structure
$R_p < 1/3$	Spherical micelles
$1/3 < R_p < 1/2$	Cylindrical
$1/2 < R_p < 1$	Bilayer
$R_p > 1$	Inverted

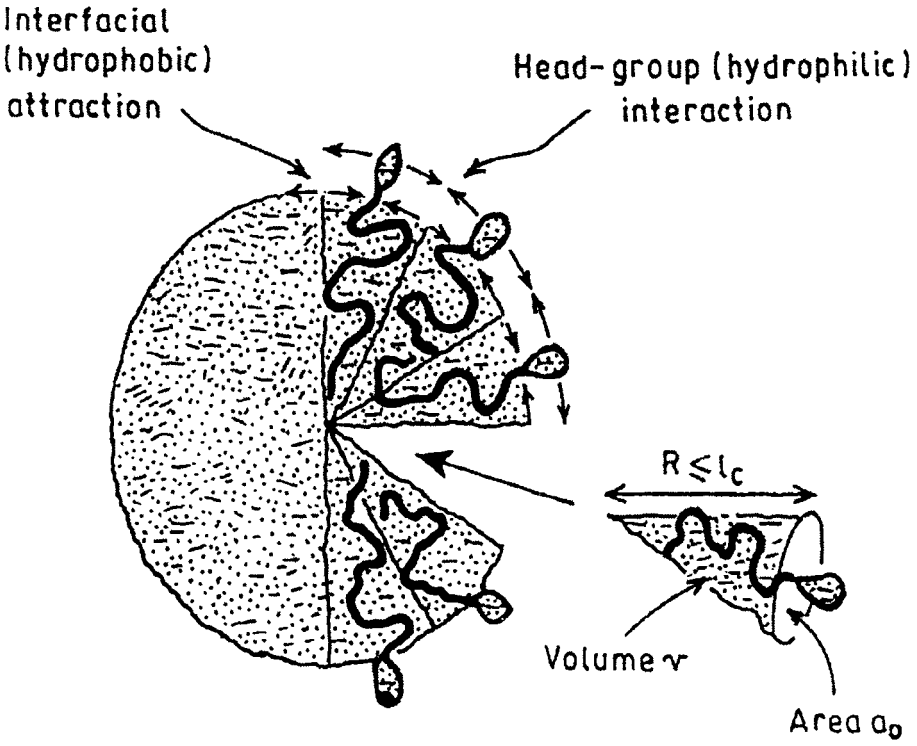


Fig. 1.2: The packing parameter (R_p) relates the head group area, the extended length and the volume of the hydrophobic part of an amphiphile molecule into the dimensionless $R_p = v/a_o l_c$.

Types of Micelles

(i) Normal micelles

The structure of a normal micelle just above the cmc can be considered as roughly spherical.[67-69] When the hydrophobic portion of the amphiphile is a hydrocarbon chain, the micelle will consist of a liquid-like hydrocarbon core. The radius of this core is roughly equal to the length of the fully extended hydrocarbon chain (~12-30 Å). The polar head groups and bound water are regularly arranged at the micellar surface, which is rough.[70] Menger has proposed that water can penetrate inside the micelle up to a certain level,[71,72] the idea gets support from fluorescence and ^{1}H–NMR measurements. Partial molar volume determinations indicate that the alkyl chains in the core are more expanded than those in the normal liquid state.[73]

The non-ionic micelles arrest water molecules at the palisade layer (which includes the head groups and the first few methylene groups) by hydrogen bonding of water with the polyethylene oxide groups.[74] Water may remain trapped in this region.

In ionic micelles, the surface potentials are high[70,75] and a significant fraction of the counterions (60-90%)[76] are located in a compact region, known as Stern layer,[70] which extends from the core to within a few angstroms of the shear surface of the micelle. The core and the Stern layer form the 'kinetic micelle'. Most of the remaining counterions are, however, located outside the shear surface in the region called 'Gouy-Chapman electrical double-layer'.

According to Hartley model, the overall volume of a micelle is approximately twice that of Stern layer.[77, 78]

Counterions are bound primarily by the strong electrical field created by the head groups but also by specific interactions that depend upon head group and counterion type.[69, 76, 79] A two-site model has been successfully applied to the distributions of counterions; i.e., they are assumed to be either "bound" to the micellar pseudophase or "free" in the aqueous phase.[80-82] The head group and counterion concentrations in the interfacial region of an ionic micelle are on the order of 3-5 M, which gives the micellar surface some of the properties of concentrated salt solutions.[75,82,83] Although the solution as a whole is electrically neutral, both the micellar and aqueous pseudophases carry a net charge because thermal forces distribute a fraction of the counterions radially into the aqueous phase.[81, 82]

(ii) Reverse micelles

Amphiphiles dissolved in non-polar solvents in presence of traces of water associate to form the so-called reverse, inverted or reverted micelles. The structure of micelle is similar to that of normal micelle but inverted, i.e., the polar head groups of the monomers being present in the center and the hydrocarbon chains extending outwards into the solvent.[84,85] Water forms a pool in the interior of the micellar aggregate. The size and properties of reverse micelles vary with the amount of water present.[86, 87]

Water in reverse micelles is expected to behave very differently from ordinary water because of extensive binding and orientation effects induced by polar heads forming the water core.[88]

(iii) Mixed micelles

Mixing of two or more amphiphiles in an aqueous solution lead to the formation of mixed micelles. A mixed micelle is an aggregate of surfactant molecules composed of different types of surfactants present in aqueous solution (Fig. 1.3).

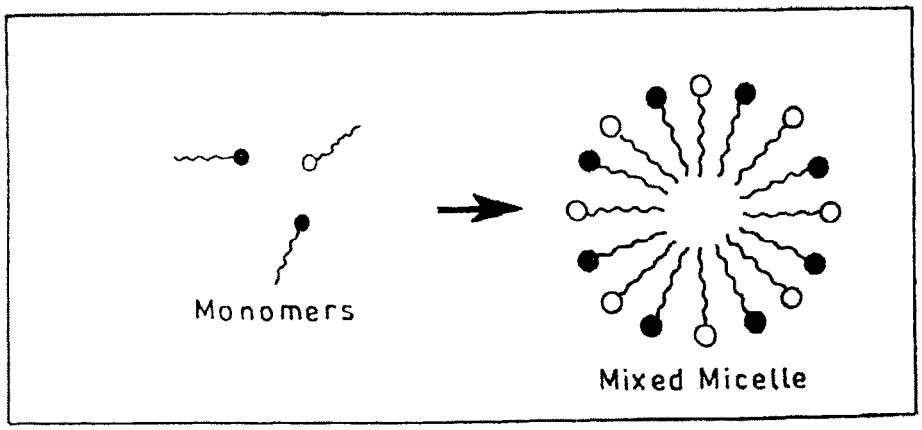


Fig. 1.3: *Schematic representation of mixed micelle.*

Mixed micelles that contain more than one type of amphiphiles are of great importance from the viewpoints of fundamental, technological, pharmaceutical and biological considerations. In practical field, mixed amphiphiles often perform better than a single amphiphile.[89-92] When two (or more) type of amphiphiles are in solution, a complex balance of intermolecular forces is responsible for the formation of mixed micelles against the formation of micelles constituted by one type of amphiphile.[92] Clint[93] developed

analytical description which contained both micelle composition and monomer concentration above the mixed cmc for mixtures of non-ionic surfactants. Clint's treatment assumed ideal mixing. The properties of the mixtures of ionic and non-ionic amphiphiles[94-96] have been interpreted with the aid of mixed micelle formation. It was pointed out that the cmc of the mixed surfactants was lower than either of the single surfactants.[93, 97]

Mixed micelles may also form when low molecular weight solutes are solubilized by micelles of amphiphiles containing a relatively larger non-polar side chain. The solubilized substances, also called as the penetrating additives,[61] may be located in both the hydrocarbon core[98] and in the hydrophilic mantle.[99-101]

Mixed micellar solutions exhibit some very interesting properties not expected from individual surfactant solution. When an ionic surfactant is mixed with a non-ionic surfactant, the degree of the association falls to zero as mole fraction of non-ionic surfactant in the micelle increases.[102,103] This is particularly evident for mixtures of anionic and non-ionic surfactants of the polyoxyethylene type, because of the strong interaction between the anionic head group and the ethylene oxide group.

Drugs and Their Classification

The term drug is derived from the French word 'Drogue" which means 'a dry herb'. According to definition of the World Health Organization, a drug is any substance or product that is used to modify or explore physiological systems or pathological states for the benefit of the recipient.[104] In the context

of medicine, it means a chemical used in the prevention, diagnosis or treatment of disease.

It is to be noted that drugs are to be used for the benefit of recipient and it is presumed that this refers to total benefit–physical, mental as well as economical. Drugs are regarded as biologically active chemical compounds mostly with a therapeutic purpose which can be broadly classified into:

(i) Biological classification (based on pharmacotherapeutic and chemotherapeutic agents)

(ii) Chemical classification (based on drugs' chemical structure)

(iii) Classification of drugs according to commercial consideration (classified according to operational expenses, research investment, and profit margins)

(iv) Classification by the lay public (classification depending on the action of the drug, like antiseptic, tonics, laxative, etc.).

A wide variety of drugs are, in fact, known to be surface active in nature.[105-112] This activity does not appear to be a fortuitous coincidence. In a number of cases excellent correlations between surface activity and biological effects have been demonstrated.[113-120]

Many pharmacologically active compounds are amphiphilic or hydrophobic molecules, which may undergo different kinds of associations and whose site of action in the organism frequently is the plasma membrane. Even if their target is intracellular, the interaction with this first barrier plays a fundamental role.[121]

Formation of cell membranes and location of receptor proteins in lipid bilayers is a consequence of surface activity. It is, therefore, logical to expect that the drugs acting by altering the permeability cell membranes after interacting with them are likely to be surface active in nature. This is because the lipid bilayers, with receptors in them, represent the interface and the drugs interacting with them will not reach the interface unless they are surface active in nature.[105]

Surface activity is of ubiquitous presence in living systems. Take any body fluid or cell soup, its surface tension is always less than that of water. Most of the biomolecules, proteins, lipids, etc. are surface active in nature. Molecules of surface active nature are crucial to living matter and its organization. Formation of biological cell is, as a matter of fact, a consequence of surface activity. Surface activity in living systems is a matter of evolution, i.e., it is need based and therefore should have a crucial role to play in biological action.[105]

Theories of drug action

There are three theories relevant to drug action namely, occupancy theory, rate theory and inactivation theory.

(i) Occupancy theory

Biological responses to drugs are, as a rule, graded; they can be measured on a continuous scale and, there is a systematic relationship between the dose of a drug and the magnitude of the response. Application of the law of

mass action to the dose-response relationship was largely done by Clark.[122,123] An observed biological effect was assumed to be a reflection of the combination of drug molecules with receptors. The magnitude of a response was postulated to be directly proportional to the occupancy of receptors by drug molecules. The maximal response is assumed to be obtained when all the receptors are occupied.

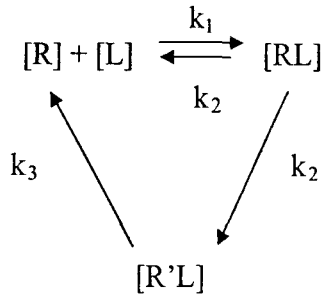
(ii) Rate theory

The central idea in this theory is different from that in the occupancy theory. Instead of attributing excitation to the occupation of receptors by drug molecules, it is attributed to the process of occupation–each association between a drug molecule and a receptor providing one quantum of excitation. The magnitude of biological response is proportional to the rate at which drug molecules associate with receptor sites. This rate depends on the concentration of free drug, the concentration of free receptor sites and the rate constants for association of drug molecules with receptor.[124-126]

(iii) *Inactivation theory*

Receptor inactivation theory is based on the two state model originally proposed by Katz and Thesleff for ion channels.[127] Kenakinn[128] on his work on the Torpedo nicotinic receptor reported that the multimeric receptor exists in active and inactive states with ligand binding altering the equilibrium between these two states. Receptor inactivation theory reflects a synthesis of both occupancy theory and rate theory providing an alternative consideration for the study of the receptor ligand interaction. Inactivation theory assumes that RL

complex is an intermediate "active state" that gives rise to an inactive form of the receptor, R', which is part of an RL complex termed R'L.[129]



where R stands for receptor and L for ligand, k_1, k_2, k_3 are corresponding rate constants.

Classes of amphiphilic drugs include analgesics,[130] antihistamines,[131] local anesthetics (LA),[132-136] tricyclic antidepressants,[137-139] phenothiazine[140-150] and benzodiazepine[151] tranquillizers,[152-155] peptide[156] and non-peptide[157,158] antibiotics,[159-161] anticholinergics,[162] β-blockers,[163] non-steroidal anti-inflammatory drugs,[164] anticancer drugs.[165] Many of these drugs contain one or more (condensed or not) aromatic nuclei, while others are of peptide nature. A great deal of data on the surface active properties of drugs can be found in the book by Attwood and Florence, [166] and other reviews.[167-169]

Surface active drugs of quite different chemical structure are reported to self-associate and bind to membranes, causing disruption and solubilization, in a surfactant-like manner.[121] Depending on the kind of drug, the self-association of these drugs classified into two modes: micellar and non-micellar aggregations. Here the micellar aggregation means that a single multimer

(micelle) forms above the cmc, and non-micellar (stepwise) aggregation means that i-mer is successively formed by aggregation of (i-l)-mer and monomer.[166]

Clouding Phenomenon in Aqueous Amphiphilic Solutions

A useful method for describing amphiphile phase behavior is in the form of a phase diagram.

When two neat liquids are brought together, they may either mix into a homogenous solution or they may form two solutions, where in each case one of the components can be regarded as the solvent. Normally in the latter case, the compositions of the two phases in equilibrium become more equal when the temperature is increased. The explanation is simple and can be expressed in terms of minimization of the free energy. From standard thermodynamics we know that

$$\Delta G = \Delta H - T\Delta S \qquad (1.6)$$

where H is the enthalpy and S the entropy. In terms of simple models such as the regular solution theory,[170-173] this is a consequence of the relative temperature independence of ΔH and ΔS. This means that the main temperature dependence comes from $T\Delta S$. This quantity is a measure of the disorder in the system, and when a mixture is formed the disorder is normally increased. Consequently, we may expect the entropy of mixing to be a positive quantity that favors mixing, and that this effect becomes more important at higher temperatures.

There are, however, frequent exceptions from this expected behavior. Such systems are represented by liquids that are completely miscible at low temperatures but phase separate when heated. This is observed by the clear solution present at low temperatures suddenly becoming cloudy on heating. The temperature where this occurs is referred to as the cloud point (CP).

Some of the more important and practical applications of micelles seem to lie in the area of separation science.[174-179] For example, aqueous micellar media have been utilized as the mobile phase additive in the layer and high performance liquid chromatography as well as the "active discriminating agent" in the electrolytic medium for electrokinetic capillary electrophoretic separations. However, extractive separation, preconcentration, and purification schemes based upon the unique phase separation behavior of aqueous solutions of amphiphilic micellar systems appear to have been largely neglected despite the demonstrated success and potential advantages of the technique compared to conventional liquid-liquid extractions.

The basis of the phase separation (or cloud-point) extraction technique, initially reported by Watanabe,[180] stems from the well-known phase phenomenon exhibited by some surfactant micellar solutions. Namely, upon appropriate alteration of the conditions (i.e., temperature or pressure change, addition of salt or other additive, etc.), the separation of an aqueous surfactant micellar solution into a concentrated phase containing most of the surfactant (termed surfactant-rich, micellar, or coacervate phase) and a dilute aqueous phase containing low concentration of surfactant is observed. Any

component(s) originally present that binds to the micellar aggregate in solution can thus be extracted from the original solution and concentrated in the small volume element of the surfactant rich-phase.

If the solution is cold, a temperature below which the amphiphile is not really very soluble (Fig.1.4) at all is reached, known as the Krafft temperature.[181-185] If, on the other hand, the temperature is raised, especially for non-ionic amphiphiles or those with some non-ionic polar groups, a two-phase region is encountered, above what is known as the CP[186-188] where two liquid (micellar) phases are in equilibrium. Finally, if we increase concentration at ambient temperature, one starts to encounter, usually at amphiphile concentration above 40% by weight, a series of mesomorphic phases sometimes called liquid crystalline phases.

Generally, the clouding behavior would not happen in ionic surfactant systems because of significant electrostatic repulsion between the charged aggregates. Nevertheless, previous researches showed that aqueous solutions of some ionic surfactants with high salt concentration,[189-192] salt-free aqueous solution of certain ionic surfactants with large head groups[193,194] or large counterions,[195-197] and some mixed cationic and anionic surfactant solutions[198] also exhibited the above behavior. The mechanism of the behavior in these ionic surfactant solutions is still an open question.[189, 199,200]

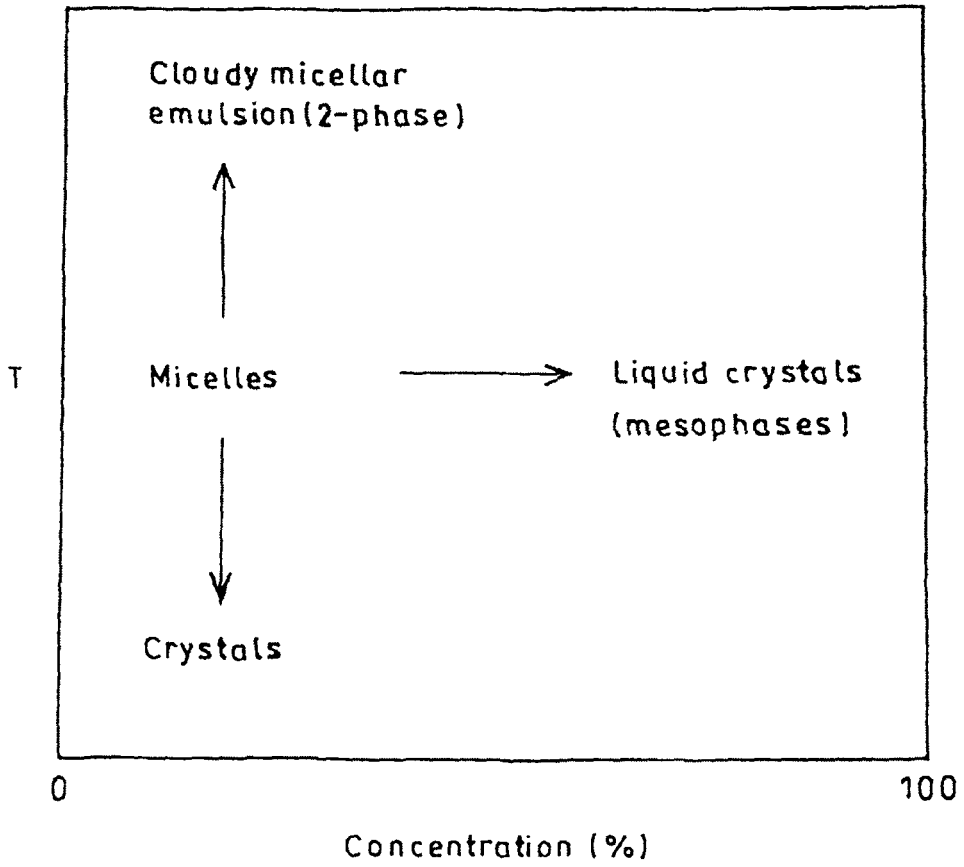


Fig. 1.4: *Schematic temperature (T)– concentration phase diagram illustrating the types of amphiphilic aggregates encountered by moving away from the micellar region.*

Mechanism for phase separation

The surfactant/salt solutions phase separate upon either heating or cooling depending on the salt type and composition. Phase separation on cooling is akin to a gas-liquid transition[201] and occur when attractive interactions between the constituent entities become sizable with respect to the thermal energy. The added salt screens the electrostatic repulsions between the surfactant micelles. An attractive potential well due to van der Waals forces can then arise between the micelles, eventually causing phase separation at low temperatures.[202] Adding more salt weakens repulsions, and the system therefore phase separates at higher temperatures.

Phase separation on heating is a more complex phenomenon and has been explored in detail previously in the context of non-ionic surfactant phase behavior. In the few cases where this phenomenon has been observed for ionic surfactant solutions, the surfactant head group was large and hydrophobic[193,194] or the salt concentrations were extremely high.[190] The results here show that neither of these factors is the key requirement, what, then, controls the phase separation on heating? Two different arguments are presented below.

(a) ***Attractive interactions between pseudo–non-ionic micelles***

The phase separation on heating would then be a natural outcome of non-ionic micellar character. This stems from the observation that clouding occurs only in the presence of hydrophobic counterions such as salicylate (Sal) and tosylate (Tos) that remain strongly bound to micelles.[203-206] These counterions are supposed to be oriented perpendicular to the micellar surface

plane, with the hydrophobic portion penetrating into the non-polar interior of the micelles and the negative charge located at the surface adjacent to the positively charged surfactant head groups.[203] The head groups and the counterions can thus form complexes at the micellar surface, and the neutralization of surface charge imparts non-ionic character to the micelles. Interestingly, such pseudo–non-ionic complexes have been found in some mixtures of cationic and anionic surfactants[192,195,207,208] and indeed these mixtures show cloud points.

The non-ionic character of the micelles will then be a function of composition and will depend especially on the fraction of salt that remains bound to the micelles.

The discussion thus far assumes that pseudo–non-ionic micelles will develop attractive interactions on heating. The molecular origin of such attractions is a matter of speculation, and it is not evident that hydrogen-bonding interactions play a role. Warr et al.[193,194] advanced a mechanism involving hydration shells to account for cloud points, whereas Appell and Porte[190] interpreted the clouding of CPB/$NaClO_3$ mixtures as being analogous to the phase separation of polymers in a poor solvent. Thus, the overall manifestation of the interactions is that water functions as a good solvent for the micelles at low temperatures and becomes a poor solvent at high temperatures.

Gaisford et al.[209] observed that copolymers with similar PO chains aggregate cooperatively, whereas mixtures with different PO blocks showed

the occurrence of independent aggregation. Zhou and co-workers[210] have elucidated the self-assembly behavior for a mixture of P105 and L64. They have observed changes in the packing features of some mesophases and, specifically for the case of micellization, they verified the occurrence of independent critical micelle temperatures.

Valauliker and Manohar[211] investigated the mechanism of clouding in the non-ionic surfactant solutions under the effect of ionic surfactant. They interpreted a linear rise in the clouding temperature of Triton X-100 upon addition of very small concentrations of SDS in terms of increase in the surface charge of the micelle.

(b) ***Microstructural changes from linear to branched micelles***

An interpretation of the observed cloud points in terms of attractive interactions ignores the dramatic microstructural changes occurring as a function of composition and temperature that are indicated by rheological measurements on erucyl bis(hydroxyethyl)methylammonium chloride (EHAC).[189] The viscosity as a function of salt (NaTos or NaSal) shows two maxima, one at low and the other at high salt concentrations. Because of the intervening two-phase region, there is a break in the curve that obscures the viscosity minimum. Other mixtures of a cationic surfactant and a binding salt (e.g., CTAB/NaSal[212] or CPC/NaSal[213]) show a continuous curve with similar viscosity maxima flanking a viscosity minimum. Thus the data are analogous, barring the phase separation at intermediate salt in the EHAC case.

Phase separation does occur at intermediate salt concentrations in some other micellar systems.[205,214,215] For 4-pentyl and 4-propyl sodium benzoates with CPC,[205] the plots of viscosity as a function of salt concentration are strikingly similar to EHAC/NaSal (no temperature dependence of the phase behavior is reported). In contrast, 4-ethyl and 4-methyl sodium benzoates with CPC do not show a two-phase region, and in the latter case, there is only a single viscosity maximum.[205] Mixtures of CTAB with low concentrations of the strong hydrotrope sodium 3-hydroxynaphthalene-2-carboxylate also separate into two phases, one of which has a lamellar morphology.[214,215]

These results suggest that highly hydrophobic counterions that can bind strongly to micelles are prone to cause phase separation even at low concentrations. In general, pronounced hydrophobicity in the salt and/or the surfactant is necessary for strong binding. In the above examples, the salt is especially hydrophobic, whereas in case of EHAC, it is the surfactant that is unusually hydrophobic because of its long (C_{22}) unsaturated alkyl chains. An analogy can thereby be drawn between phase separation occurring in CPC/ 4-pentyl sodium benzoate and EHAC/NaSal, whereas pairs of relatively less hydrophobic surfactant and salt such as CPC/NaSal remain a single phase over the range of compositions.

The link between the rheology and the phase transitions can be further rationalized if they are both being driven by the same microstructural changes. Specifically, the first viscosity maximum might signify a shift from linear to branched micelles,[216,217] as a connected network of branched micelles will

have a lower viscosity than an entangled network of linear micelles.[217] Recent theories suggest that, as branching proceeds, the system might eventually phase separate into a saturated micellar network (i.e., a branched network with no free ends) and a dilute surfactant solution.[199,200,218] The driving force for phase separation is the entropic attraction between network junctions.[199]

For occurrence of clouding in ionic micellar solutions, Yu and Xu [195] proposed another mechanism for tetrabutyltetradecyl sulfate. They postulated that butyl chains belonging to $TBuA^+$ associated with one micelle could cross-link to another micelle helping overcome the effects of electrostatic repulsion and an energetic barrier due to oriented water near the surface of the two micelles. To be operative geometrically, it appears that the two micelles would have to approach one another intimately due to the limited extent of the short butyl chains.

Bales and Zana[197,219–221] have shown reservations towards the mechanism for clouding in TBuADS (or SDS $+TBuA^+$) as being due to reducing the water hydrating the micelle[222-226] and said measurements of the water content of the palisade layer will be needed to confirm this expectation.[227] If indeed clouding is induced somehow by dehydrating the micelle surface, then the interpretation of the data will be further complicated by presence of Na^+ because increasing the aggregation number of a globular micelle leads to less water per surfactant.[219,227] This is a consequence of the fact that the surface area of a micelle grows more slowly than its volume, leading to

a smaller volume per surfactant in which to fit the water.[219,227] Adding Na^+ increases the aggregation number of SDS. Therefore, there would be two competing mechanisms to dehydrate: one by displacement of water by the counterions and another by the geometric constrictions due to micelle growth. Therefore, one would need to know the details of the micelle aggregation numbers in the presence of both $TBuA^+$ and Na^+ to gain a clear understanding.

To the previously suggested mechanisms for clouding in ionic micelles, they suggested [221] that a second layer of $TBuA^+$ is loosely attached outside the polar shell of the TBuADS micelle because steric restrictions did not appear to allow enough available volume to house a sufficient number of counterions. If this second layer is in fact available, the cross-linking between micelles could take place between butyl groups of the $TBuA^+$ ions in the second layer. This possibility is supported by the tendency of $TBuA^+$ ions to self-associate.[228-232]

So far, most of these studies on clouding behavior in ionic micellar solutions were made in systems where $TBuA^+$ was added either externally or was part of the surfactant monomer.[195-197,222-226] Also, the variation of head group from tripropyl- to tributylammonium in a cationic surfactant caused the observance of clouding in solutions on heating.[193,194] These results suggest the crucial roles played by temperature and alkyl chains present near the head group region in dictating the macroscopic properties of the surfactant solutions. The above studies justify a need to know details of micellar morphologies that lead to clouding.

In a series of papers Kabir-ud-Din and his group[222-226,233] have reported that adding salts with large hydrophobic cations to anionic surfactants can lead to clouding when such behavior is absent in the pure surfactants. A mechanism of clustering of the micelles as the temperature approaches the CP is proposed in ionic surfactants having tetraalkylammonium counterions ($\geq TBuA^+$).[234] The clustering is due to depletion of H-bonded water present around the alkyl chains at the micellar surface. The size of the clusters seems to depend upon the length of the hydrocarbon chain present in the quaternary counterion and the temperature.

Recently, Kim and Shah[235-237] have observed the CP phenomenon in amphiphilic drug amitriptyline hydrochloride solutions and have explored the effect of additives. Also, Kabir-ud-Din and his group[238-240] have studied CP phenomenon in some amphiphilic drug solutions and examined the effects of different additives.

Relevance of the Problem

The motivation for studying the solution properties of amphiphilic drugs is:

(i) although the pharmacological effects of amphiphilic drugs are usually manifest at concentration well below the critical micelle concentration (cmc), it is likely that accumulation of drug molecules in certain sites in the body may cause a localized high concentration resulting in aggregation and consequent

changes in biological activity due to decreased transport rates or decreased ability to pass through biological barriers.[166,241,242]

(ii) drug molecules exert activity by interaction with biological membranes, this membrane affinity being a measure of the hydrophilic-hydrophobic interactions in the molecule that can be related to the surface activity of the drugs.

These drugs, because of their amphiphilic nature, exhibit concentration, temperature and pH-dependent phase separation.[235-240] It was observed that CP can vary with additives. When using these drugs it should be kept in mind that normal human body temperature is typically 12 degrees above ambient. Even if the CP of pure drug in buffer is above this temperature, it may decrease in presence of additives, especially surfactants which are used as drug-carriers. As clouding concentrates the drug in a small volume, it may affect the activity of drugs and, therefore, it is important to have a knowledge of clouding behavior of these drugs in designing more effective drug-carrier combinations. With this idea in mind, effect of various additives, viz., electrolytes, non-electrolytes, alcohols, amino acids, sugars, surfactants and their concentration effects have been examined on CP and dye solubilization behavior of some amphiphilic drugs.

Layout of the Thesis

This thesis consists of four chapters including this one which is concerned mainly with the general introduction of amphiphiles, the factors responsible for the formation of various aggregation patterns, their solution properties, e.g., cloud point phenomenon, etc. From the literature survey it appears that exploring CP in amphiphilic drugs in presence of additives is a recent phenomenon. Experimental details are provided in **Chapter II**.

Chapter III contains cmc data for two antidepressant (nortriptyline, NOT, and clomipramine, CLP) and phenothiazine (promazine, PMZ) drugs in aqueous media. Effect of some selected additives on the cmc of these drugs in aqueous solutions is also reported.

Studies on the effect of various additives on the CP values and dye solubilization of two antidepressant (NOT, CLP) and phenothiazine (PMZ) drugs are described in **Chapter IV**.

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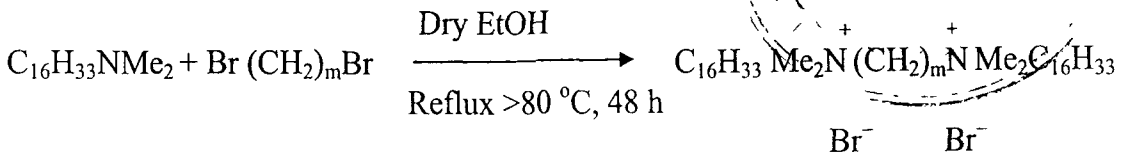
Chapter–II

Experimental

The materials used throughout the study are given in Table 2.1, including their abbreviated names, chemical formulas/structures, sources, and purities.

All the drugs as well as additives (electrolytes, ureas, sugars, surfactants, amino acids and alcohols) were used as received.

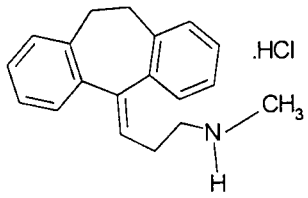
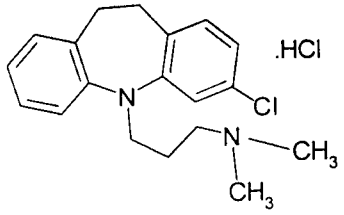
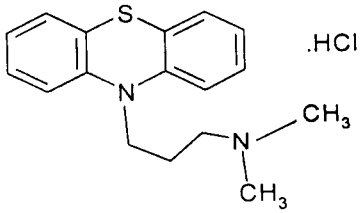
Gemini surfactants were synthesized in the laboratory by the following method[1]



(m = 4, 5)

A 2.1:1 equivalent mixture of *N*, *N*-dimethylhexadecylamine with corresponding α, ω-dibromoalkane (m = 4, 5) in dry ethanol was refluxed (at 80 °C) for 48 h. After completion (TLC technique was used to monitor the progress of the reaction), the solvent was removed under vacuum from the reaction mixture and the solids thus obtained were recrystallized several times from hexane/ethyl acetate mixtures to obtain the compounds in pure form. The overall yields of the surfactants ranged from 70 to 90%. Purity of all the gemini surfactants was checked on the basis of C, H, N analysis, which was further characterized by ^{1}H NMR.[1, 2]

Table-2.1: Names and structural formulas of the chemicals used.

Name	Abbreviation	Structure / Formula	Make	% Purity
(a) Amphiphilic Drugs:				
Nortriptyline hydrochloride ($pK_a = 9.4$)[a]	NOT		Sigma (USA)	≥ 98
Clomipramine hydrochloride ($pK_a = 9.4$)[a]	CLP		Sigma (USA)	≥ 98
Promazine hydrochloride ($pK_a = 9.4$)[a]	PMZ		Sigma (USA)	≥ 98

Contd....

(b) Surfactants:

Sodium dodecyl sulfate	SDS	$CH_3(CH_2)_{11}OSO_3^-Na^+$	Sigma (USA)	99
Sodium dodecylbenzene sulfonate	SDBS	$CH_3(CH_2)_{11}C_6H_4\ SO_3^-\ Na^+$	TCI (Japan)	≥ 99
Cetyltrimethylammonium bromide	CTAB	$CH_3(CH_2)_{15}N^+(CH_3)_3\ Br^-$	BDH (England)	> 99
Tetradecyltrimethylammonium bromide	TTAB	$CH_3(CH_2)_{13}N^+(CH_3)_3\ Br^-$	Sigma (USA)	99
Cetylpyridinium chloride	CPC	$CH_3(CH_2)_{15}N^+C_5H_5\ Cl^-$	BDH (England)	≥ 98
Cetylpyridinium bromide	CPB	$CH_3(CH_2)_{15}N^+C_5H_5\ Br^-$	E. Merck (Germany)	≥ 99
Polyoxyethylene sorbitan monolaurate	Tween 20	—	LOBA Chemie (India)	—
Polyoxyethylene sorbitan monopalmitate	Tween 40	—	Koch–Light (England)	—
Polyoxyethylene sorbitan monostearate	Tween 60	—	LOBA Chemie (India)	—

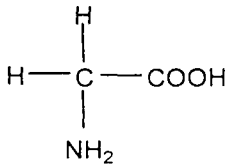
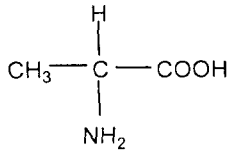
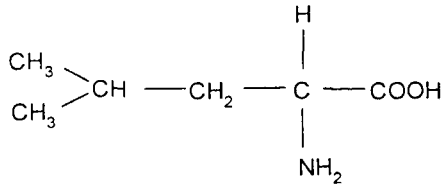
Contd....

Butanediyl–α, ω– bis (N–hexadecyl–N, N-dimethylammonium bromide)	16-4-16	$C_{16}H_{33}(CH_3)_2N^+(CH_2)_4N^+-(CH_3)_2C_{16}H_{33}\ 2Br^-$	Self synthesized	—
Pentanediyl–α, ω– bis (N-hexadecyl–N, N-dimethylammonium bromide)	16-5-16	$C_{16}H_{33}(CH_3)_2N^+(CH_2)_5N^+-(CH_3)_2C_{16}H_{33}2Br^-$	Self synthesized	—
(c) Electrolytes:				
Lithium chloride	LiCl	—	Merck (Germany)	≥ 99
Lithium bromide	LiBr	—	Riedel-deHaen (Germany)	≥ 99.4
Sodium fluoride	NaF	—	BDH (England)	≥ 97
Sodium chloride	NaCl	—	BDH (England)	≥ 99.9
Sodium bromide	NaBr	—	LOBA Chemie (India)	≥ 99
Potassium chloride	KCl	—	BDH (India)	≥ 99.8
Potassium bromide	KBr	—	Merck (India)	≥ 99
Tetramethylammonium bromide	TMeAB	$(CH_3)_4NBr$	Fluka (Switzerland)	≥ 97

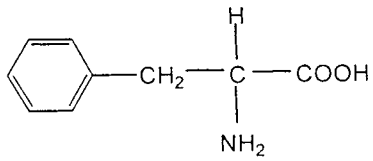
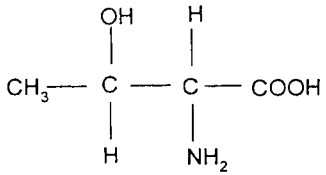
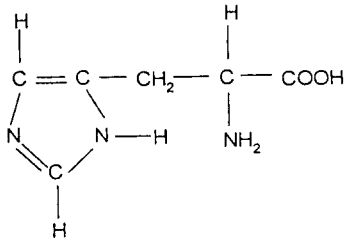
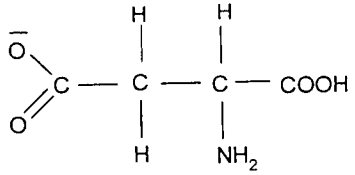
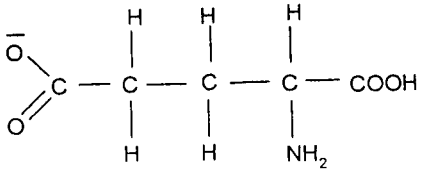
Contd

Tetraethylammonium bromide	TEtAB	$(C_2H_5)_4NBr$	Fluka (Switzerland)	≥ 98
Tetra-*n*-propylammonium bromide	TPrAB	$(n\text{–}C_3H_7)_4NBr$	Fluka (Switzerland)	≥ 98
Tetra-*n*-butylammonium bromide	TBuAB	$(n\text{–}C_4H_9)_4NBr$	Fluka (Switzerland)	≥ 98

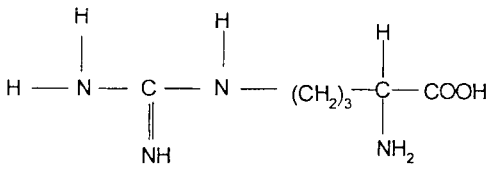
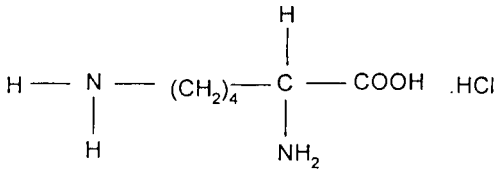
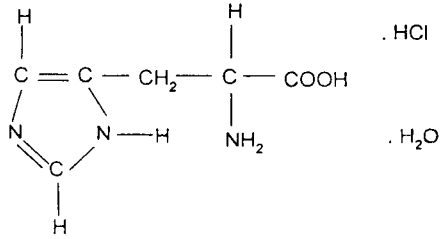
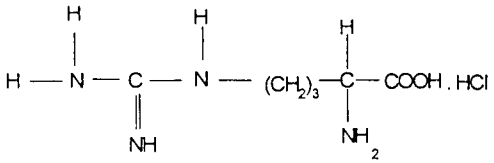
(d) Amino acids:

Glycine		SISCO (India)	> 99.5
β-Alanine		SISCO (India)	>99
L-Leucine		Merck (Germany)	>98

Contd...

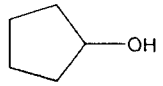
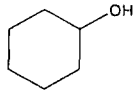
L(-)-Phenylalanine		Merck (Germany)	>99
DL-Threonine		BDH (England)	>98.5
L-Histidine		LOBA Chemie (India)	>99
DL-Aspartic acid		SISCO (India)	>99
L-Glutamic acid		SISCO (India)	>99

Contd...

L-Arginine		Fluka (Switzerland)	≥ 99.5
L-Lysine monohydrochloride		Fluka (Switzerland)	≥ 98
L-Histidine monohydrochloride monohydrate		BDH (England)	≥ 98.5
L-Arginine monohydrochloride		LOBA Chemie (India)	>99

Contd...

(e) Alcohols:

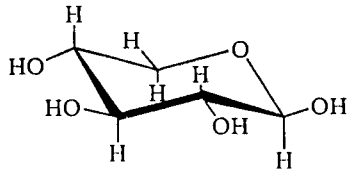
Ethanol	C_2OH	C_2H_5OH	Merck (Germany)	99.8
Propanol	C_3OH	$CH_3(CH_2)_2OH$	BDH (England)	99.9
Butanol	C_4OH	$CH_3(CH_2)_3OH$	Sarabhai (India)	99.9
1–Pentanol	C_5OH	$CH_3(CH_2)_4OH$	Fluka (Switzerland)	>99
1–Hexanol	C_6OH	$CH_3(CH_2)_5OH$	BDH (England)	>99
1–Heptanol	C_7OH	$CH_3(CH_2)_6OH$	BDH (England)	>99
1–Octanol	C_8OH	$CH_3(CH_2)_7OH$	Fluka (Switzerland)	>97
Ethanediol		CH_3 $(OH)CH_2OH$	BDH (England)	>99
Propan-1, 2-diol		CH_3 CH (OH) CH_2 OH	BDH (India)	>99
Cyclopentanol			Fluka (Switzerland)	≥ 98
Cyclohexanol			BDH (India)	>98
Allyl alcohol		$H_2C=CHCH_2$ OH	Duchem (India)	>95

Contd...

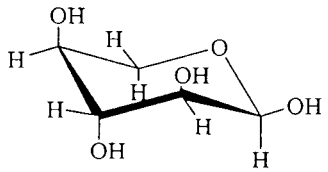
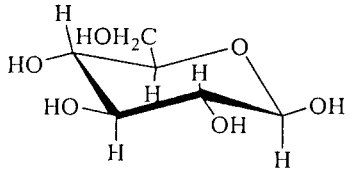
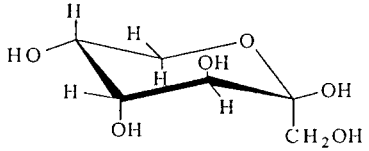
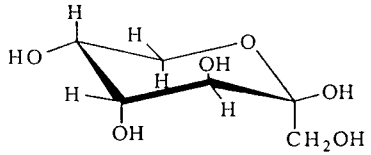
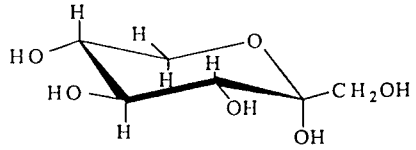
(f) Ureas:

Urea	U	CH_4N_2O	Sigma (USA)	>99.5
N-Methylurea	MU	$C_2H_6N_2O$	Merck (Germany)	>97
N, N-Dimethylurea	DMU	$C_3H_8N_2O$	Sigma (USA)	≥98
Tetramethylurea	TMU	$C_5H_{12}N_2O$	Fluka (Switzerland)	≥ 99
Thiourea	TU	CH_4N_2S	Sigma (USA)	>99
N, N-Dimethylthiourea	DMTU	$C_3H_8N_2S$	Lancaster (England)	≥98

(g) Sugars:

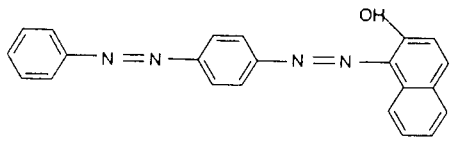
D(+)Xylose			s.d. fine (India)	99

Contd....

L(+) Arabinose		Fluka (Switzerland)	99
D(+)Glucose		Merck (India)	99
D(+)Mannose		s.d. fine (India)	99
D(–)Fructose		Merck (India)	99
L(–)Sorbose		Fluka (Switzerland)	98

Contd....

(h) Dye:

Sudan III			LOBA Chemie (India)	—

(i) Sodium phosphate salts:

Sodium dihydrogen phosphate monohydrate	MBSP	$NaH_2PO_4.H_2O$	Merck (India)	≥ 99
Trisodium phosphate dodecahydrate	TBSP	$Na_3PO_4.12H_2O$	Merck (India)	≥ 98

(j) Acids:

Sulfuric acid		H_2SO_4	Merck (India)	98
Hydrochloric acid		HCl	Merck (India)	35-37

[(a)]The pK_a values of the drugs in free molecular state: B. G. Katzung, Basic and Clinical Pharmacology, 9th ed., McGraw Hill: New York, 2004.

Hygroscopic chemicals (drugs: NOT, CLP, all quaternary salts, LiCl, LiBr, etc.) were stored in desiccators. PMZ was kept in a refrigerator (at 4 °C). NOT is not only hygroscopic but also photosensitive, so it was stored in desiccator at a dark place (wrapped in aluminium foil).

All solutions were prepared in doubly-distilled deionized water. The specific conductivity of the water was in the range 1-2 x 10^{-6} S cm^{-1}.

Special care was taken for cleaning the glasswares, which were properly washed with freshly prepared chromic acid and distilled water then rinsed with acetone and kept in oven for drying before use.

Preparation of Sodium Phosphate Buffer Solutions:

10 mM sodium phosphate (SP) buffer solution was prepared from SP monobasic monohydrate (X mM) and SP tribasic dodecahydrate (Y mM) and subsequently used throughout as solvent for CP measurements.[3-5] (For NOT: X = 7.0, Y = 3.0 ; for PMZ: X = 5.0, Y =5.0 for CLP: X = 7.0,Y = 3.0).

For the pH variation, the 10 mM SP buffer ratios of X and Y were:

X = 9.5. Y = 0.5; X = 9.0, Y = 1.0; X = 8.0, Y = 2.0; X = 7.0, Y = 3.0; X = 6.0, Y = 4.0; X = 5.0, Y = 5.0; X = 5.5, Y = 4.5.

Cloud Point Measurements:

To determine the CP, the sample solution was taken in a securely stoppered Pyrex glass tubes which were then placed in a controlled stirring and heating device. The

temperature was slowly raised. The onset of sudden clouding in the solution was taken as CP.[6–9] The heating was discontinued until the sample become clear again. The temperature was cycled (at least twice) in this way to obtain the CP temperature (reproducibility; ± 0.5 °C). Similar CP measurements were made by using different [additive] at fixed [drug] (50 mM except with [NOT] = 30 mM due to the limited solubility of the drug). This was done by diluting the sample to smaller concentrations and repeating the same procedure. These experiments were performed to obtain the minimum [drug] required to observe the clouding phenomenon.

Dye Solubilization Experiments:

The term solubilization implies the formation of thermodynamically stable isotropic solution of a substrate (the solubilizate), normally insoluble or only slightly soluble in a given solvent, by the addition of an amphiphile (the solubilizer).[10] Solubilization is of course, closely related to micellization since little or no solubility increase is observed until the cmc of the amphiphile is reached, but once the micelles are fully formed its increase is directly proportional to the concentration of the amphiphile over a large range.

Dye solubilization experiments were performed at room temperature by vigorously stirring 20 cm^3 of drug solutions with 20 mg of Sudan III dye for 5 min, then separating the insoluble dye by filtration. The visible spectra (wavelength range: 430-650 nm) were recorded on a Shimadzu UV 1240 mini UV-visible spectrophotometer.

Conductivity Measurements:

Conductivities were measured with an ELICO conductivity bridge, model CM82T. The cell containing solution was immersed in a water bath keeping the temperature control to ±0.1°C. The temperature was kept constant at 30 °C. Stock solutions of drug and additive were prepared in double distilled water and then desired mole fraction was prepared by mixing pre-calculated volumes of stock solutions. The conductivity was recorded by successive addition of concentrated stock solution in water. A break in the conductivity vs. total concentration curve signaled the onset of the micellization process.

pH Measurements :

An ELICO pH–meter type LI–120 fitted with an ELICO CH–41 glass and calomel combination electrode was used for pH-measurements. The electrode was stored in pH 7 buffer and was washed in deionized double–distilled water before use. It was then rinsed with pH 7 buffer solution and the pH–meter was standardized using pH 4 buffer solution. Whenever the solution was changed, the electrode was rinsed with double–distilled water and the surplus water was removed and pH–meter was restandardized using the pH 4 buffer solution. All pH–measurements were made at least in triplicate and they agreed within ± 0.02.

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Chapter–III

Critical Micelle Concentration of Amphiphilic Drugs with and without Additives

Micelle formation of an amphiphile in solution is induced by the hydrophobic interaction between hydrocarbon parts of the amphiphile balanced by the hydration of electrostatic repulsive effect of their head groups.[1] All supramolecular assemblies produced by self-association of amphiphiles are characterized by various parameters such as critical micelle concentration (cmc), aggregation number and shape; of which cmc is the most predicted and easily determined parameter.

The cmc is the narrow range of concentration over which amphiphile solutions show an abrupt change in physical properties. The cmc can, therefore, be determined by the intersection of two straight lines of a variety of solution properties above and below the cmc.

Typical aggregation behavior is shown by a large number of drugs from many pharmacological groups like local anesthetics, antibiotics, tranquillizers and antidepressants. Many workers have reported the association behavior of pencillins,[2] phenothiazines,[3] antidepressants,[4] etc.

The tricyclic antidepressant and phenothiazine drugs possess an almost planar ring system with a hydrocarbon chain and a charged nitrogen atom. These drugs form small aggregates of 6-12 monomers in solution.[5, 6] The pK_a values of these drugs lie between 9.1–9.4[7] and depending upon solution pH, the drug molecules may become protonated or neutral. These drugs form mixed micelles with surfactants.[8] Drug-surfactant mixed micelles are pharmaceutically important as undesirable side effects of these drugs may be reduced if used as mixed micelles. Also, surfactants can act as drug carriers and

enhance the drug's solubility. Therefore, it is important to have knowledge of the effect of surfactants on the cmc of amphiphilic drugs.

With this view point conductometric studies have been performed on aqueous solutions of three amphiphilic drugs, nortriptyline hydrochloride, NOT; clomipramine hydrochloride, CLP (antidepressants); and promazine hydrochloride, PMZ (phenothiazine) to determine the effect of surfactants and NaCl on the cmc of these drugs. Two conventional surfactants CTAB, TTAB and two gemini surfactants, 1,5-pentanediyl-α-ω-bis (N-hexadecyl-N, N-dimethylammonium bromide), 16-5-16; 1,4-butanediyl-α, ω-bis (N-hexadecyl-N, N-dimethylammonium bromide),16-4-16 were used. The compositions of drug-surfactant mixed micelles are estimated using Rubingh's theory.

Results and Discussion

Representative plots of specific conductivity, κ, as a function of total amphiphile concentration are shown in Figs. 3.1-3.4. Similar plots were obtained for other concentrations. In all plots, κ shows a clear break corresponding to the cmc. The cmc is obtained from the intersection of the two straight lines above and below this break.

In aqueous medium, the amphiphile solutions of low concentration behave as simple electrolyte solutions and most of the amphiphile molecules exist as free monomers. However, as the surfactant concentration is increased, solution behavior changes. Beyond the cmc, most of the amphiphile exists in

the micellar form and the total monomer concentration becomes practically constant. Therefore, the gradient of conductivity decreases after cmc.

The cmc values for pure drugs have been found to be in good agreement with the literature values,[9,10] whereas the values decrease in the presence of additives (NaCl, surfactants). Added counterions (inorganic salts) are bound to micelles primarily not only by the strong electrical field created by the head groups, but also by specific interactions that depend upon the head group and the counterion type. Two mechanisms have been proposed: in one mechanism, inorganic salts affect the solvent property of water while in another, ions affect the micelles directly by adsorbing/desorbing to the head group region of the micelles.[11,12] Counterion binding plays a role to decide the effective charge on the micelles and hence its formation, shape, and mutual interactions.[13] Added Cl^- ions (in the form of NaCl) follow the second mechanism and adsorb to the cationic head group region of the drug monomers. This adsorption decreases the electrostatic repulsion among head groups and less electrical work is required to form micelles. As a result, the cmc decreases (Table 3.1).

Similar to the behavior of individual amphiphiles on micellization, mixed systems undergo changes in solution properties. Mixed micelles possess physicochemical properties distinctly different from those of pure micelles of the individual components. Most cmc's of binary mixtures fall in between the cmc's of pure components but some are above [14] or below [15] this range.

Tables 3.2- 3.4 present the values of experimental cmc, ideal cmc, mole fraction of additives in micelles, their ideal values along with activity coefficients f_1 and f_2 of both components–drugs and surfactants.

At the mole fractions studied, cmc decreases with the addition of conventional as well as gemini surfactants. This suggests that mixed micelles are formed in the solution and the surfactants are penetrating into the micelles formed by the drug molecules. With gemini surfactants, the decrease is sharper than with conventional surfactants. As gemini surfactants contain two hydrophobic chains, they increase the hydrophobic interactions and enhance the tendency of drugs to form micelles to a larger extent than the conventional surfactants. Due to the difference in hydrophobic portion of drugs and surfactants, the mixed micelles would show non-ideality. For ideal mixing, Clint's model[16] relates the mole fraction, α_i, and cmc of pure micelles of component i, cmc_i, through the equation:

$$\frac{1}{cmc^*} = \sum_i \frac{\alpha_i}{cmc_i} \tag{3.1}$$

cmc*(ideal cmc) values come out to be higher than the experimental cmc values. Hydrophobicity of drug molecules as well as that of surfactants causes micellization to occur at concentrations lower than expected for ideal mixing.

Several parameters are used to study the interactions between the drug and surfactant molecules in their mixed state. The mole fraction of surfactant (X_1) in the mixed micelles is calculated according to the following equation:[17, 18]

$$\frac{X_1^2 \ln [cmc\alpha / cmc_1 X_1]}{(1-X_1)^2 \ln[cmc(1-\alpha)/cmc_2(1-X_1)]} = 1 \qquad (3.2)$$

where cmc = cmc of the mixed system,

cmc_1 = cmc of the surfactant, and cmc_2 = cmc of the drug.

Eq. (3.2) is solved iteratively to obtain the value of X_1.

The micelle mole fraction in the ideal state X_1^{id} has been computed by Motomura's approximation:[19]

$$X_1^{id} = \frac{\alpha cmc_2}{[\alpha cmc_2 + (1-\alpha)cmc_1]} \qquad (3.3)$$

The results in Tables 3.2-3.4 show that both X_1 and X_1^{id} increase as α increases. This increase in X_1 indicates that mixed micelle formation is favored compared to micelle formation of pure components and mixed micellar phase is enriched in drug molecules. Also, X_1^{id} is smaller than X_1 suggesting that more surfactant is present in micellar phase than it should be in ideally mixed state. Large hydrophobic volume of drug molecules makes it difficult for them to associate and hence in a micellar aggregate less drug molecules are present than what it would have been on the basis of ideal mixing.

The nature and strength of interaction between the components of the mixture can be determined by calculating the value of an empirical parameter, β,[17] which is calculated by the following equation:

$$\beta = \frac{\ln (cmc\alpha / cmc_1 X_1)}{(1-X_1)^2} \qquad (3.4)$$

The β value is assumed to be constant for a particular system. According to Rubingh,[17] a negative β value indicates an attractive interaction or synergism in the system, a positive value indicates a repulsive interaction or antagonism and an almost nil value means no interaction or ideal mixing. Also, the higher the magnitude of β the greater would be the interaction whether attractive or repulsive.

The β values in this study, although not constant, show strong synergism with average values for the three drugs with 16-5-16, 16-4-16, CTAB and TTAB, respectively, as:

(i) NOT: -4.65, -4.08, -5.61, and -6.57,

(ii) CLP: -4.32, -4.33, -5.60, and -6.49,

(iii) PMZ: -5.49, - 4.99, -6.70, and -8.23.

The added surfactants assist drug molecules in micelle formation which is also clear from decrease in cmc.

The activity coefficients, f_1 (of the drug) and f_2 (of the surfactant), are directly related to both β and X_1 by the following relations: [20]

$$f_1 = \exp\{\beta (1-X_1)^2\} \qquad \text{and} \tag{3.5}$$

$$f_2 = \exp\{\beta X_1^2\} \tag{3.6}$$

Increase in interactions between various molecules in the mixed systems changes the activity coefficients of the components and deviates them from unity. As can be seen (Tables 3.2-3.4), f_1 and f_2 are lower than unity.

Excess free energy of mixing, ΔG_{ex}, is related to f_1 and f_2 by

$$\Delta G_{ex} = RT\,[X_1 \ln f_1 + (1-X_1)\ln f_2] \qquad (3.7)$$

where R = gas constant, and T = temperature in Kelvin scale.

The ΔG_{ex} values are all negative which is self-explanatory in the light of above discussion. The drug-surfactant mixed micelles are more stable than the micelles of pure drug.

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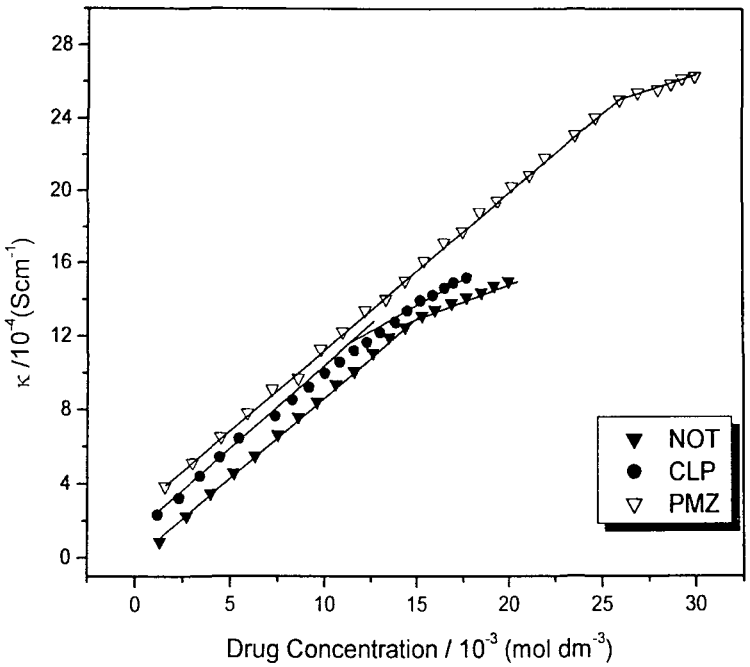

Fig. 3.1: *Plots of specific conductivity* (κ) *vs. drug concentration for solutions containing 100 mM NaCl. The scale shown is for NOT. Other curves have been shifted upwards by 1, 2 scale units, respectively.*

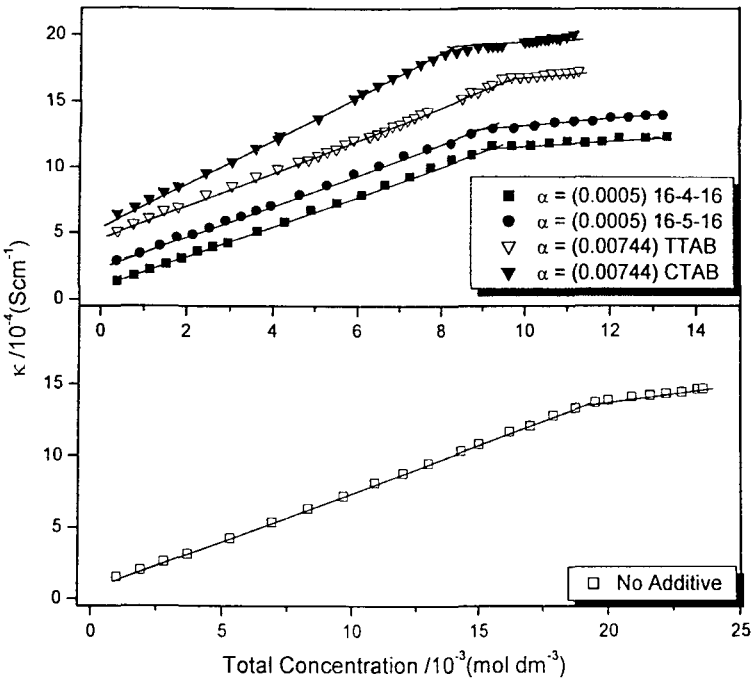

Fig. 3.2: *Plots of specific conductivity of surfactant+ NOT mixtures vs. total concentration at some selected mole fractions (α). The scales shown are for no additive and 16-4-16. Other curves have been shifted upwards by 1, 2, 3 scale units, respectively.*

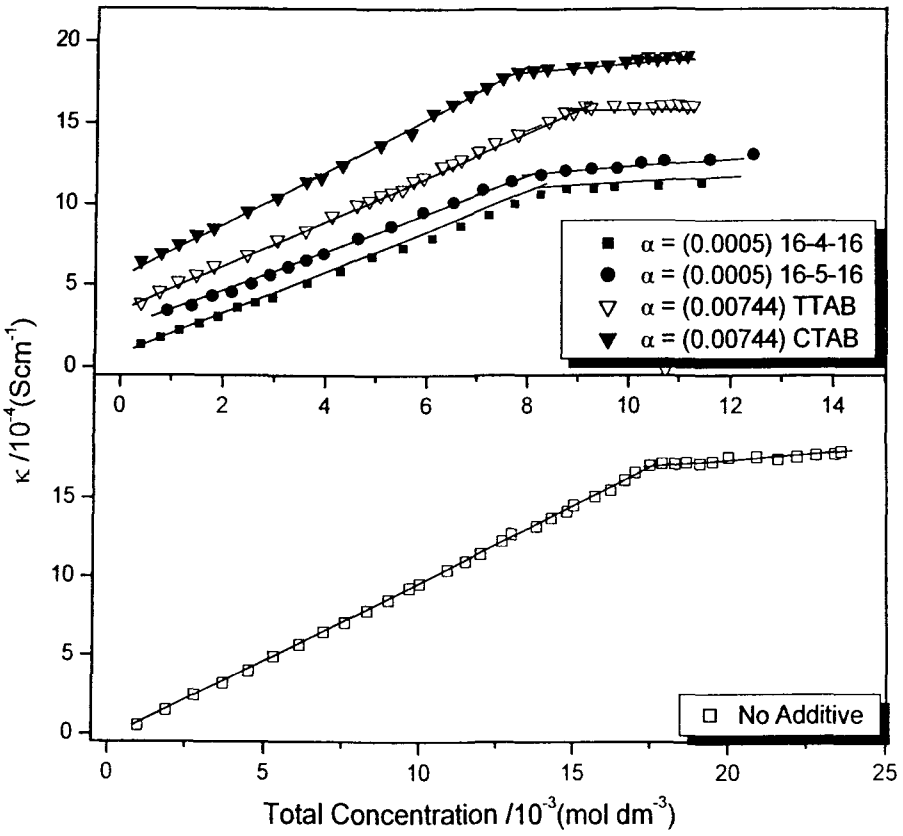

Fig. 3.3: *Plots of specific conductivity of surfactant+ CLP mixtures vs. total concentration at some selected mole fractions (α). The scales shown are for no additive and 16-4-16. Other curves have been shifted upwards by 1, 2, 3 scale units, respectively.*

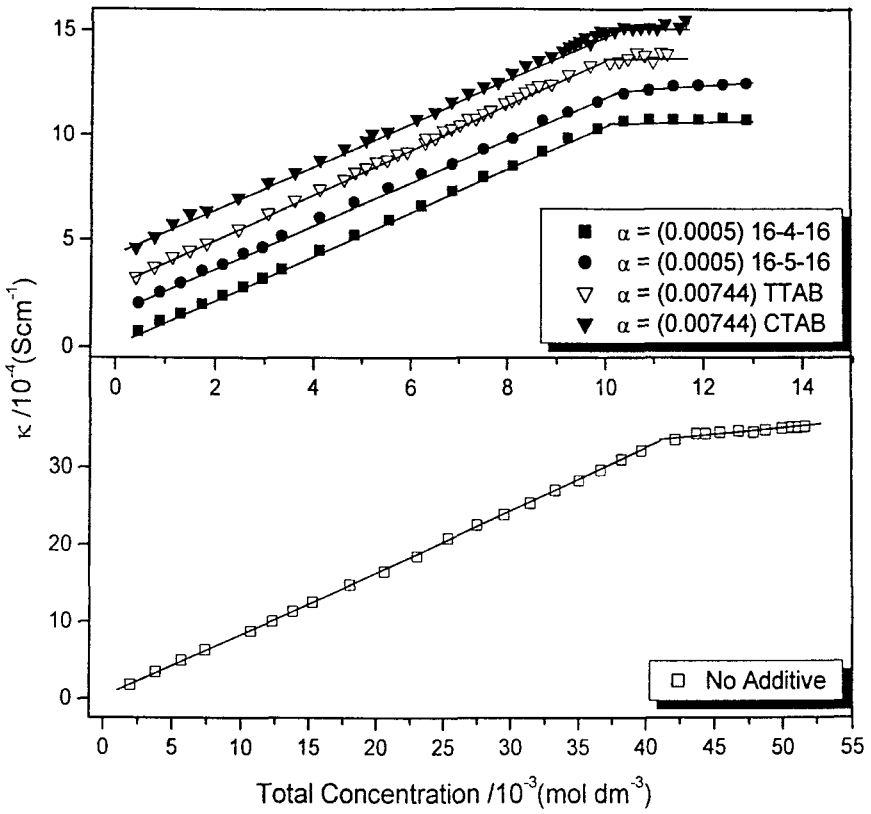

Fig. 3.4: *Plots of specific conductivity of surfactant + PMZ mixtures vs. total concentration at some selected mole fractions (α). The scales shown are for no additive and 16-4-16. Other curves have been shifted upwards by 1, 2, 3 scale units, respectively.*

Table 3.1: Effect of NaCl concentration on the cmc values of amphiphilic drug solutions.

[NaCl]/mM	NOT cmc/mM	CLP cmc/mM	PMZ cmc/mM
0	19.33	17.82	41.03
50	16.54	14.83	-
100	14.48	11.66	26.52
150	13.30	9.70	-
200	9. 77	6.57	23.12
300	-	-	21.51
400	-	-	17.40

Note. Uncertainties on cmc are estimated to be less than $\pm 1 \times 10^{-4}$.

Table 3.2: Experimental cmc (cmc), ideal cmc (cmc*), micellar composition (X_1, X_1^{id}), interaction parameter (β), activity coefficients (f_1, f_2), and excess free energy (ΔG_{ex}) of binary mixtures of NOT with surfactants at different mole fractions of surfactants (α).

α	cmc/mM	cmc*/mM	X_1	X_1^{id}	β	f_1	f_2	ΔG_{ex} (J mol^{-1})
				16-5-16				
0	19.33	-	-	-	-	-	-	-
0.00022	11.00	17.30	0.285	0.105	-2.826	0.236	0.795	-1450.60
0.00050	9.08	15.25	0.356	0.211	-2.502	0.354	0.728	-1470.14
0.00076	3.94	13.75	0.439	0.289	-5.286	0.189	0.361	-3282.98
0.00110	1.70	12.17	0.474	0.371	-7.991	0.109	0.166	-5028.55
				16-4-16				
0	19.33	-	-	-	-	-	-	-
0.00022	11.54	16.71	0.287	0.136	-2.190	0.328	0. 835	-1130.39
0.00050	9.20	14.24	0.373	0.264	-1.993	0.457	0.758	-1174.02
0.00076	3.70	12.52	0.457	0.352	-5.011	0.228	0.352	-3131.18
0.00110	1.83	10.82	0.487	0.441	-7.131	0.153	0.184	-4493.20

Contd...

				CTAB				
0	19.33	-	-	-	-	-	-	-
0.0050	12.33	17.71	0.254	0.089	- 2.544	0.243	0.849	-1213.44
0.0074	8.31	17.01	0.338	0.127	-3.867	0.183	0.644	-2180.97
0.0120	4.90	15.84	0.403	0.190	-5.401	0.146	0.419	-3274.09
0.0200	1.09	14.15	0.464	0.283	-10.640	0.047	0.101	-6672.93
				TTAB				
0	19.33	-			-	-	-	-
0.0050	14.16	18.97	0.182	0.024	-3.483	0.097	0.891	-1308.13
0.0074	9.81	18.79	0.271	0.035	-5.077	0.067	0.689	-2530.70
0.0120	5.58	18.48	0.345	0.055	-7.053	0.049	0.432	-4008.07
0.0200	1.97	17.95	0.409	0. 090	-10.668	0.024	0.168	-6501.77

Note. Uncertainties on cmc are estimated to be less than $\pm 1\times 10^{-4}$.

Table 3.3: Experimental cmc (cmc), ideal cmc (cmc*), micellar composition (X_1, X_1^{id}), interaction parameter (β), activity coefficients (f_1, f_2), and excess free energy (ΔG_{ex}) of binary mixtures of CLP with surfactants at different mole fractions of surfactants (α).

α	cmc/mM	cmc*/mM	X_1	X_1^{id}	β	f_1	f_2	ΔG_{ex} (J mol^{-1})
				16-5-16				
0	17.82	-	-	-	-	-	-	-
0.00022	10.90	16.08	0.266	0.098	-2.573	0.250	0.833	-1267.43
0.00050	7.91	14.30	0.359	0.198	-2.882	0.306	0.689	-1673.28
0.00076	5.58	12.97	0.414	0.273	-3.660	0.284	0.534	-2240.04
0.00110	1.57	11.56	0.471	0.352	-8.163	0.102	0.164	-5120.33
				16–4–16				
0	17.82	-	-	-	-	-	-	-
0.00022	11.2	15.56	0.271	0.127	-2.048	0.337	0.860	-1020.02
0.00050	8.30	13.40	0.370	0.248	-2.213	0.415	0.739	-1300.41
0.00076	4.40	11.87	0.444	0.334	-4.130	0.279	0.443	-2569.46
0.00110	1.13	10.33	0.486	0.421	-8.921	0.095	0.122	-5608.63

Contd...

				CTAB				
0	17.82	-	-	-	-	-	-	-
0.0050	12.52	16.43	0.224	0.082	-2.117	0.280	0.899	-926.92
0.0074	7.80	15.84	0.332	0.117	-3.909	0.175	0.650	-2183.74
0.0120	3.90	14.47	0.407	0.110	-6.151	0.115	0.361	-3741.42
0.0200	1.15	13.33	0.459	0.267	-10.228	0.050	0.116	-6402.91
				TTAB				
0	17.82	-	-	-	-	-	-	-
0.0050	14.00	17.51	0.157	0.022	-3.087	0.112	0.927	-1027.34
0.0074	8.82	17.37	0.273	0.032	-5.320	0.060	0.673	-2661.43
0.0120	4.64	17.11	0.350	0.051	-7.632	0.040	0.393	-4369.52
0.0200	2.26	16.67	0.400	0.083	-9.907	0.028	0.205	-6001.22

Note. Uncertainties on cmc are estimated to be less than $\pm 1\times 10^{-4}$.

Table 3.4: Experimental cmc (cmc), ideal cmc (cmc*), micellar composition (X_1, X_1^{id}), interaction parameter (β), activity coefficients (f_1, f_2), and excess free energy (ΔG_{ex}) of binary mixtures of PMZ with surfactants at different mole fractions of surfactants(α).

α	cmc/mM	cmc*/mM	X_1	X_1^{id}	β	f_1	f_2	ΔG_{ex} (J mol $^{-1}$)
				16-5-16				
0	41.03	-	-	-	-	-	-	-
0.00022	11.90	32.83	0.397	0.200	-4.668	0.183	0.479	- 2817.93
0.00050	10.20	26.17	0.453	0.363	-3.885	0.313	0.451	-2423.99
0.00076	6.81	22.02	0.490	0.464	-4.817	0.285	0.314	-3039.20
0.00110	2.14	18.24	0.511	0.556	-8.598	0.128	0.106	-5413.66
				16-4-16				
0	41.03	-	-	-	-	-	-	-
0.00022	12.00	24.59	0.412	0.251	-4.160	0.237	0.494	-2540.11
0.00050	10.16	23.32	0.475	0.432	-3.360	0.396	0.469	-2110.87
0.00076	7.10	19.05	0.513	0.538	-3.975	0.390	0.351	-2502.54
0.00110	1.91	15.36	0.525	0.627	-8.461	0.148	0.097	-5321.12

Contd...

				CTAB				
0	41.03	-	-	-	-	-	-	-
0.0050	14.20	34.19	0.375	0.171	-4.261	0.189	0.549	-2519.23
0.0074	10.81	31.61	0.413	0.235	-4.748	0.195	0.445	-2899.55
0.0120	6.20	27.72	0.458	0.333	-6.187	0.162	0.273	-3874.60
0.0200	1.26	22.79	0.494	0.456	-11.622	0.051	0.059	-7314.67
				TTAB				
0	41.03	-	-	-	-	-	-	-
0.0050	15.00	39.22	0.320	0.094	- 6.136	0.059	0.534	-3357.85
0.0074	10.93	38.39	0.358	0.071	-6.959	0.057	0.409	-4031.45
0.0120	6.13	36.93	0.403	0.111	-8.662	0.046	0.245	-5243.82
0.0200	2.69	34.63	0.441	0.173	-11.169	0.031	0.114	-6920.56

Note. Uncertainties on cmc are estimated to be less than $\pm 1\times 10^{-4}$.

Chapter–IV

Clouding and Dye Solubilization in Amphiphilic Drug Solutions

Non-ionic surfactants generally[1-3] and ionic surfactants in special conditions[4-7] undergo clouding phenomenon. Amphiphilic drugs, like ionic surfactants, undergo pH, concentration and temperature dependent phase separation.[8-13] It was further observed that CP can vary with additives. Many pharmacologically active compounds are amphiphilic, they tend to self-associate as micelles in aqueous solutions and to interact with biological membranes, causing disruption and solubilization in a surfactant-like manner.[14] Drug self-association depend on the molecular structure of the drug, its concentration, pH, temperature and additive concentration.[15-18] Aggregates of these amphiphilic drugs could act as their own carriers. It has been postulated that the drug vesicle formation is also feasible.[19] However, most drugs are not lipophilic enough to form vesicles and hence require drug delivery systems to administer them into the body and to help control the site of delivery. For drug delivery it is essential that the drug carrier must not be mistaken for invading microbes otherwise they will rapidly be mopped up by microphages. Over the years, therefore, micelles have been of interest to pharmacological scientists either as drug delivery systems or as targeting systems.[20] Both cases have the advantage of protecting the body from side effects of the drug at the same time attaining the required concentration of the drug at the site.

When using these drugs it should be kept in mind that normal human body temperature is typically 12 degrees above ambient. Even if the CP of pure drug in

buffer is above this temperature, it may decrease in presence of additives, especially with surfactants (which are used as drug carriers).

At CP the drug concentrates into a small volume, leading to localized high concentration at particular site. This may lead to aggregation causing a change in biological activity due to decreased ability to pass through biological barrier[15] and may prove harmful.

Human blood plasma normally has a pH close to 7.4. Should the pH-regulating mechanisms fail, as may happen in severe uncontrolled diabetes because of the acidosis caused by over production of metabolic acids, the pH of the blood can fall to 6.8 or below and can lead to irreparable damage and death.[21] And in other diseases, the pH may rise to the point of on return. These observations indicate that pH effect should be taken into account when dealing with a drug solution. Investigations on the effect of additives and experimental pH on the clouding behavior of amphiphilic drugs were started fairly recently.[8-13]

An important property of micelles that has a particular significance in pharmacy is their ability to increase the solubility of sparingly soluble substances.[22,23] A number of approaches have been taken to measure solubilizing behavior of amphiphiles in which the solubilization of water insoluble dye in the surfactant micelles was studied.[24]

Amphiphilic drugs, as mentioned before, form small aggregates above their cmc and may act as their own carrier in drug delivery. However, water-insoluble

or sparingly soluble drugs need carriers for their safe transport to the desired site. Over the years, several of the phase structures produced by surfactants were of interest to the pharmaceutical scientists, either as drug vehicles/carriers or, recently, as targeting systems. Non-ionic surfactants are widely investigated as a means of producing a clear stable solution of poor water-soluble drug suitable for intravenous or oral administration.

Hence, clouding behavior and dye solubilization of amphiphilic drugs in aqueous buffer solutions were examined by investigating the following factors: (1) pH, (2) nature and concentration of externally added salts, (3) addition of ureas and thioureas, (4) addition of sugars, (5) addition of amino acids, (6) addition of alcohols, and (7) addition of cationic (conventional and gemini), anionic and non–ionic surfactants.

Results and Discussion

The drug concentrations considered in the studies described below are above their respective critical micelle concentrations (Chapter III, Tables 3.1-3.4) where drug micelles start to form in the aqueous solution.

(A) Effect of pH

As already mentioned in the Introduction, occurrence of CP phenomenon in amphiphilic systems is the resultant of the interplay of hydrophobic and hydrophilic forces. Additives, which are known to ameliorate these forces, are bound to modulate the system's CP. With this view point we now begin with the

effect of pH on the CP of drug solutions. The results are shown in Figs. 4.1 and 4.2. Both in the absence and presence of additives, the CP decreases with the increase in pH range employed. This is due to deprotonation of nitrogen atom of *tert*-amine portion of the drug molecules. This effect reduces the micellar surface charge and electrostatic repulsion among monomers which, in turn, increases the aggregation number and compactness of micelles. Consequently, clouding takes place at lower temperatures. The behavior remains the same in the presence of additives; the changes in CP values (increase or decrease with respect to the no additive case) being dependent upon the nature of additive.

Figs. 4.3 and 4.4 illustrate the influence of pH on the visible spectra of Sudan III in drug solutions. Increase in solution pH results in increased absorbance which indicates that the size of the micelles is increasing. Increase in pH leads to deprotonation of nitrogen atom of tertiary amine portion of the drug molecule. This deprotonation increases intermicellar compactness due to a decrease in head group repulsion and consequent increase in micellar size. The resulting micelles dissolve more drugs and hence the absorbance increases with increasing pH.

(B) Effect of electrolytes

(i) inorganic salts

Effect of added counterions (F^-, Cl^-, Br^-, in the form of their sodium salts) on the CP of drug solutions are illustrated in Figs. 4.5 and 4.6. The CP increases in the presence of the above salts. Figs. 4.7 and 4.8 show the visible spectra of Sudan

III in 30, 50, and 75 mM-NOT, CLP, and PMZ, respectively, micellar solutions at 100 mM salt concentrations. It can be seen that the absorbance increases as one goes from NaF to NaBr. From the results of Figs. 4.5-4.8, the effect of counterion binding is found to be in the order Br^-> Cl^-> F^-. Ions can be classified into Hofmeister or lyotropic series according to their effect on water structure and salting-in/-out effects.[25] Two mechanisms have been given to explain their behavior:

(i) large, singly charged ions with low charge density, appearing on the right hand side of the series, are water structure breakers and called chaotropes (e.g., SCN^-, I^-, etc.), whereas small ions with high charge density (at the left hand side of the series) are water structure makers and called kosmotropes (e.g., OH^-, F^-, Cl^-, etc.).[26]

(ii) adsorption and desorption of ions to the head group of the amphiphiles cause salting-in and salting-out phenomena.[27] Further, substitution of one counterion by another also affects the interaction between counterion and surfactant, which may change the extent of binding or ionization, and consequently change the properties of ionic surfactants.[28]

As halide ions carry opposite charge, they should interact electrostatically with the micelles and affect the size/shape of the micelles.[29] Fluoride ions bind weakly to the cationic head group of the micelles as F^- is highly hydrated and, therefore, micelle size/shape changes slowly. Increase in CP and visible intensity

is thus slow in presence of NaF. With the increase in size, ion hydration decreases. Due to their smaller hydrated radius (as compared to F^-) Cl^- or Br^-can come closer to the micelle surface and thus better screen the charge of monomers. Hence, micellar growth takes place in presence of these counterions. However, these ions are also hydrated, although less than F^-, and at the same time increase the hydration of the micelles. Therefore, CP increase is sharp with these ions. The CP and visible absorbance increase with micellar growth has been proposed by Kim and Shah.[8] They observed large increase in CP as well as visible intensity with amitriptyline solution with the addition of NaCl or NaBr.

Figs. 4.9 and 4.10 show the visible spectra of Sudan III in the drug micellar solutions at three NaCl concentrations. The absorbance increase with increasing NaCl concentration indicates increase in the dye solubility. Addition of electrolytes raises the aggregation number of ionic micelles due to electrostatic effects as proposed on several occasions.[29,30] The presence of counterion (Cl^-) decreases the surface area occupied per drug head group (a_o) without affecting l_c and v (length and volume of the drug monomer, respectively). Hence Mitchell-Ninham parameter, $R_p = v/a_o l_c$, increases and micellar growth takes place.[31,32]

Similar CP increasing trend is observed with the co-ions. As shown in Figs. 4.11 and 4.12, the order of CP increase being $Li^+ < Na^+ < K^+$. In this series, Li^+ is highly hydrated (crystal radius: 0.60Å, hydrated radius: 3.28Å) while K^+ is least hydrated (crystal radius: 1.33Å, hydrated radius: 3.31Å).[33] Thus, Li^+ decreases the

availability of water to the micelles which results in a slow increase in CP compared to the increase with K^+ or Na^+ (the extent of decrease of availability of water to the micellar head group region, as per their crystal radii, is $K^+ > Na^+ > Li^+$).

(ii) quaternary ammonium bromides

Figs. 4.13 and 4.14 show the variation of CP with the addition of quaternary ammonium bromides (QABs). These salts are water structure makers and their ability to enhance water structure decreases with increase in the salt's alkyl chain length. At first sight, it seems that TBuAB should increase the CP more than TMeAB. However, the efficacy is opposite being TBuAB < TPrAB < TEtAB < TMeAB. The CP increase may be explained due to adsorption or mixed micelle formation of these salts with the drug molecules. As the hydrophobicity of the salt increases with the chain length, these salts adsorb to the drug micelle or form mixed micelles, removing water and dehydrating the micelles. Therefore, CP increase becomes slower with the increase in the length of the alkyl part of the salt.

The visible spectra of Sudan III in the drug solutions containing quaternary ammonium bromides are shown in Figs. 4.15 and 4.16. One can see that the absorbance increases on QAB addition. The quaternary salts raise the aggregation number of micelles due to mixed micelle formation. The observed increase in absorbance indicates increased dye solubility in the larger mixed micelles.

(C) Effect of organic compounds

Aqueous solutions of amphiphilic compounds have a general tendency to solubilize a certain amount of organic additives. The environment of solubilization of different additives in or around micelles can be correlated with their structural organizations and mutual interactions. Both dynamic and structural properties of micellar solutions can be altered by additives which can act through two different mechanisms: by interactions with the surfactant molecules or by changing the solvent nature.[34] Therefore, the effect of addition of organic compounds depends on as how they change the water structure and the micelle structures. The emerging picture is that molecules with polar groups are mainly solubilized near to the surface of the micelle with their polar group at the surface and that aliphatic hydrocarbons are preferentially solubilized in the micellar core. The effect of water-soluble compounds can be explained by considering their influence on the water structure.

(i) ureas and thioureas

Variation of CP of drug solutions with added ureas/thioureas are shown in Figs. 4. 17 and 4.18. Thioureas increase the CP whereas ureas decrease it. It is well known that urea decreases the polarity of micellar interface.[35,36] In order to explain the effect of urea on micellar properties, two mechanisms have been proposed. [37-39] In the first mechanism, urea affects only the solvent changing the water structure and promoting the dissolution of hydrophobic species (the so-

called *indirect* mechanism). The other one is *direct* in which urea replaces some of the water molecules from the hydration shell. The first mechanism is the most widely accepted one and urea is considered as water structure breaker.[40,41] However, recent results suggest that only small differences are present in the properties of water in the solvation region of urea and bulk water.[42-44] Our results with urea also support the direct mechanism. Due to the cationic nature of drug micelles, urea-micelle interaction is ion-dipole type. Urea interacts with micelle head group and removes water from the interfacial region; this dehydration causes a decrease in CP

Figs. 4.17 and 4.18 data also show that the rate of CP decrease increases as the number of methyl groups increase in urea. Inclusion of mono-, di- or tetramethyl groups in urea increases the size of alkyl ureas and therefore, more water is replaced by their addition with the result that the CP decrease becomes progressively more pronounced, i.e., the order of effectiveness is U < MU < DMU < TMU. The opposite effect of thiourea may be due to the difference in nature of >C= S and >C= O bonds: the >C= O is stronger than >C= S (as O is more electronegative). Therefore, electrons around S atom will be delocalized, thus making the S-atom electron deficient, i.e., a Lewis acid. As a result, the S-atom in thiourea would behave like a positive center, and repulsion between thiourea and drug micelle could be responsible for the observed CP increase.

The effect of added ureas (100 mM urea and thiourea) on the spectra of Sudan III in the drug solutions are illustrated in Figs. 4.19 and 4.20. In the case of urea one can see that the absorbance decreases indicating decreased solubility of the dye. On the other hand, the absorbance increases on thiourea addition. The results favorably support that the addition of non-electrolytes (urea/thiourea) decreases/increases the micellar size.

(ii) sugars

Figs. 4.21 and 4.22 show the effect of sugar addition on the CP of drug solutions. Figs. 4.23 and 4.24 illustrate the visible spectra of Sudan III in the presence of sugars. One can see that the absorbance decreases indicating decreased solubility of the dye. All the sugars lowered the CP. These observations are similar in form to the decrease in the water solubility of hydrophobic derivatives caused by sugars and can be explained by considering the enhanced hydrophobic interactions.[45] The CP depression indicates a 'salting-out' effect because the temperature range in which single phase solutions prevail is reduced.[46] The sugars remove water molecules surrounding the micelles and help the micelles to approach each other easily. It was suggested by Kjellander and Florin[47] that appearance of cloud point is entropy dominated. When the sugars are added, the water of hydration of the micelles decreases, as these additives compete for water molecules associated with the micelles. Thus, with two relatively less hydrated micelles approaching each other, the hydration spheres overlap and some of the

water molecules are set free to increase the entropy of the system. At cloud point, the water molecules get totally detached from the micelles. In any case, the overall entropy is high and hence the free energy change is relatively more negative and the appearance of CP is facile.[48]

The visible spectra of Sudan III in the drug solutions containing sugar (Figs. 4.23 and 4.24) are similar to that of urea case (Figs. 4.19 and 4.20). Apparently the adsorption of non-electrolytes such as urea and sugars at the micelle-water interface originates a restriction of micellization and is responsible for a decrease in the visible absorbance. Seemingly the oxygen atoms in the added non-electrolytes (urea/sugar) interact with the drug head groups, which may reduce the micellar size and in turn lower the CP.

(iii) amino acids

Results of adding different amino acids on the CP of drug solutions are shown in Figs. 4.25 and 4.26. Sharp CP increase with the acidic amino acids but non-polar and uncharged polar amino acids remaining much less effective, the nature and molecular structure of the amino acid seemingly play a role. The negatively charged side chain of acidic amino acids would interact with tertiary amine of the drug. This will allow further hydration of the micelles, hence the CP of the system increases. Hydrophobic non-polar and uncharged polar amino acids, on the contrary, would partition in micellar interior or bulk water, respectively. In either case the hydration of micelles is not affected and the CP as well.

Figs. 4.27 and 4.28 illustrate the effect of basic amino acids and their hydrochloride salts on the drug CP's. The basic amino acids, being polar, partition in the head group region with the result that certain amount of water near the head group region is replaced; the observance of CP phenomenon at a lower temperature is justified this way. Hydrochloride salts bear a positive charge on them and their interaction with the drug micelles would result in increased micelle–micelle repulsion. The CP would then increase which indeed is the case (Figs. 4.27 and 4.28).

(iv) alcohols

Figs. 4.29 and 4.30 show the effect of aliphatic alcohols on the CP of drug solutions. Short chain alcohols increase the CP slightly while longer chain alcohols decrease it sharply. Short chain alcohols are completely miscible with water and, in micellar solutions, only a small fraction solubilizes in micelles. The addition of these alcohols always results in a decrease in the aggregation number.[49] The micelles even disappear when enough alcohol is added to the micellar solution.[50] These short chain alcohol molecules may be adsorbed preferentially at the micelle-water interface.[51] Since most of them are placed outside the micelles, they would hinder the micellar aggregation. Therefore, CP increases slowly in presence of these alcohols.

Long chain alcohols are only partially soluble in water and, being increasingly solubilized by the micelles, they are expected to affect the micelle

size. There are two factors affecting the micellar growth: one is the electrical repulsion originating from inter- and intra-micellar repulsions which favors micelles with a high surface area per head group, the other is due to the hydrophobic interactions between the hydrocarbon parts of monomers which try to form tightly packed aggregates. Longer chain alcohols solubilize in the micelles with their hydroxyl groups toward the surface. The effect of these alcohols on the micellar growth can be explained by taking into consideration the Mitchell-Ninham packing parameter, R_p (= $v/a_o l_c$) Penetration of alkyl chain of alcohols will increase the volume of hydrophobic part of drug molecule (v). Also, the intercalation of –OH group between the charged head groups reduces the repulsion among them which leads to a decrease in area per head group (a_o). Consequently, R_p value increases[32] and the CP decreases due to the formation of larger size micelles.

Figs. 4.31 and 4.32 show the effect of added diols on the CP of drugs. With ethanediol and propane–1,2–diol, CP remains almost constant. Diols, being hydrophilic and highly miscible in water (as they contain two–OH groups on hydrophilic ethane or propane molecules), remain in aqueous phase at all concentrations and would not affect the micelle hydration. However, cycloalkanols show different behavior; with allylalcohol and cyclopentanol, unlike cyclohexanol, the CP decrease is not pronounced in the beginning. The results are

in conformity to the relative solubility of the cycloalkanols and prove cyclohexanol to be the highest on hydrophobicity scale in the present system.[52]

(D) Effect of surfactants

(i) cationic surfactants

Figs. 4.33 and 4.34 illustrate the effects of cationic surfactants (conventional as well as geminis), wherein we always find CP increase in presence of these surfactants. The magnitude of CP increase has been found to depend upon the following characteristics of the cationic surfactants used:

(a) length of the hydrophobic tail,

(b) nature of the head group,

(c) nature of the counterion,

(d) gemini *vs.* conventional, and

(e) gemini spacer length.

The added cationic surfactants exist in the drug solutions as monomers, micelles and mixed micelles that depend on their cmc values. Addition of the cationic surfactants to the micellar drug solutions produces larger mixed micelles that generate stronger electrostatic repulsion among the micelles and produce a higher CP. Since the head group is same in both CTAB and TTAB, the result of a greater degree increase in CP with the former indicates that the hydrophobicity of the surfactant has a role to play toward the overall CP phenomenon. Further, CPB

is found to be more effective than the CTAB. Seemingly, in addition to the hydrophobic interactions (like CTAB– both the surfactants are C_{16} type), the π electronic cloud of the aromatic nucleus interacts with the drug micelle head groups through a cationic–π effect. Obviously, this will enhance micellar growth and result in larger micelles with CP occurring at higher temperatures. The addition of a bromide surfactant (CPB) increases the CP more than a chloride surfactant (CPC). As Br^- ion has stronger binding effect than Cl^-, the presence of the former as a counterion is responsible for the decrease in surface area occupied per drug head group (a_0), with a simultaneous increase in the Mitchell-Ninham parameter, R_p [31, 32] An increase in R_p results in micellar growth producing higher CP. Figs. 4.33 and 4.34 also show the effect of gemini surfactants on drug solutions. The addition of gemini surfactants (a gemini surfactant of the type 16-m-16 in which the spacer is a hydrophobic polymethylene chain – $(CH_2)_m$– (m = 4 and 5)) provides higher CP's than the conventional cationic surfactants. The CP of gemini surfactant-drug systems also increases with increase in the surfactant concentration; the increase following the order with m-value 4 < 5. It has been shown that the surface charge increases with the increase in spacer chain length and significantly influences the aggregation properties of these surfactants.[53,54] Therefore, repulsion is higher with the higher m. This results in a faster increase in CP with the added 16-5-16 than with 16-4-16. Moreover, the geminis (due to having two tails) are more hydrophobic than their conventional single tail counterparts. The higher CP's obtained with the geminis substantiate the point.

Another view point can be put forth here. It has been found that increasing the m-value produces gradually loose micelles. Thus, less compact (relatively loose) micelles will show CP occurrence at higher temperatures.

(ii) anionic surfactants

Effect of anionic surfactants (SDS and SDBS) on the CP of 50 mM drug solutions is shown in Figs. 4.35 and 4.36. At the experimental pH, the drug monomers are assumed to be in protonated form, and drug aggregates would be positively charged. The surfactants should interact with drug micelles both hydrophobically and electrostatically and should decrease the CP. However, the results in Figs. 4.33 and 4.34 are unusual as, with the increasing surfactant concentration, the trend evidenced is:

increase ⟶ maximum ⟶ decrease. At low concentrations, these surfactants hinder micellar association (possibly by ion-pair formation) and hence the CP increases.[10] Above a certain added surfactant concentration, the decrease in CP of the system can be explained by considering the nature of alkyl chain and charge on the head group of surfactant monomers. Addition of surfactant decreases the head-head repulsion among drug molecules which, in turn, decrease the surface area occupied per monomer (a_o). Also, interaction of the surfactant alkyl chain with the hydrophobic portion of the drug strengthens the hydrophobic interactions among the hydrophobic moieties of the drug. This increases the effective volume of the micelles (v). Both of these factors lead to an increase in

R_p, which means micellar growth and formation of compact micelles that decreases the CP.

We see that the CP decrease is sharper with SDBS in comparison to SDS; this difference in behavior is understandable in view of their relative basicity ($-SO_3^- > -OSO_3^-$).[55] Further, presence of a benzene ring in SDBS increases the hydrophobicity of monomers, which is reflected in lower cmc value for SDBS.[56] Also, the presence of a benzene ring causes greater electrostatic interaction with the cationic drug molecules. All of these factors make SDBS more effective in dehydrating the drug micelles, causing a sharper decrease in CP at higher relative concentration.

(iii) non-ionic surfactants

The results of CP variation of drug solutions with added non-ionic surfactants (Tween 20, 40, 60) are depicted in Figs. 4.37 and 4.38 The CP always increases upon addition of these surfactants. As these non-ionic surfactants contain oxyethylene chains and hence are hydrophilic in nature; therefore, the drug-surfactant mixed micelles are more hydrated. Consequently, more heating is required to dehydrate these mixed micelles and hence clouding occurs at higher temperatures.

Figs. 4.39 and 4.40 illustrate the visible spectra of Sudan III in drug solutions containing different surfactants. One can see that the absorbance increases on surfactant addition. Addition of surfactants raises the aggregation

number of micelles due to mixed micelle formation. Increased dye solubility in the larger mixed micelles is responsible for the observed increase in absorbance. The results of CP increase with added cationic CTAB and TTAB support the explanation (see Figs. 4.33 and 4.34).

(E) Miscelleneous

(i) effect of additives at different fixed pHs

Figs. 4.41–4.46 illustrate the variation of CP of 50 mM (30 mM for NOT) drug solutions at different fixed pHs prepared in 10 mM SP buffer. Additive concentration variations at different fixed pHs show that increase in pH causes a decrease in CP. The drug molecules in micelles become deprotonated with increase in pH, which reduces the inter-micellar electrostatic repulsion. This enhances the association of drug micelles, leading to a decrease in CP. At any fixed concentration of additives, a decrease in CP is observed with increased pH due to deprotonation of drug micelles/monomers. This causes lowering of the electrostatic repulsion (among micelles) with a concomitant larger decrease in CP.

(ii) effect of additives at different fixed drug concentrations

The effect of additives at different fixed drug concentrations is shown in Figs. 4.47–4.52. As expected, the values of CP are higher for higher drug concentrations. The number, size, and charge of micelles increase with the increase in the drug concentration, which increases both inter-micellar and intra-micellar repulsions, causing increase in the CP value.

Figs. 4.53 and 4.54 illustrate the visible spectra of Sudan III in solutions of different fixed drug concentrations (without additive). The absorbance increase with increasing drug concentration indicates increase in the dye solubility. Obviously, the number and size of micelles increase with the increase in the drug concentration and hence the dye solubility.

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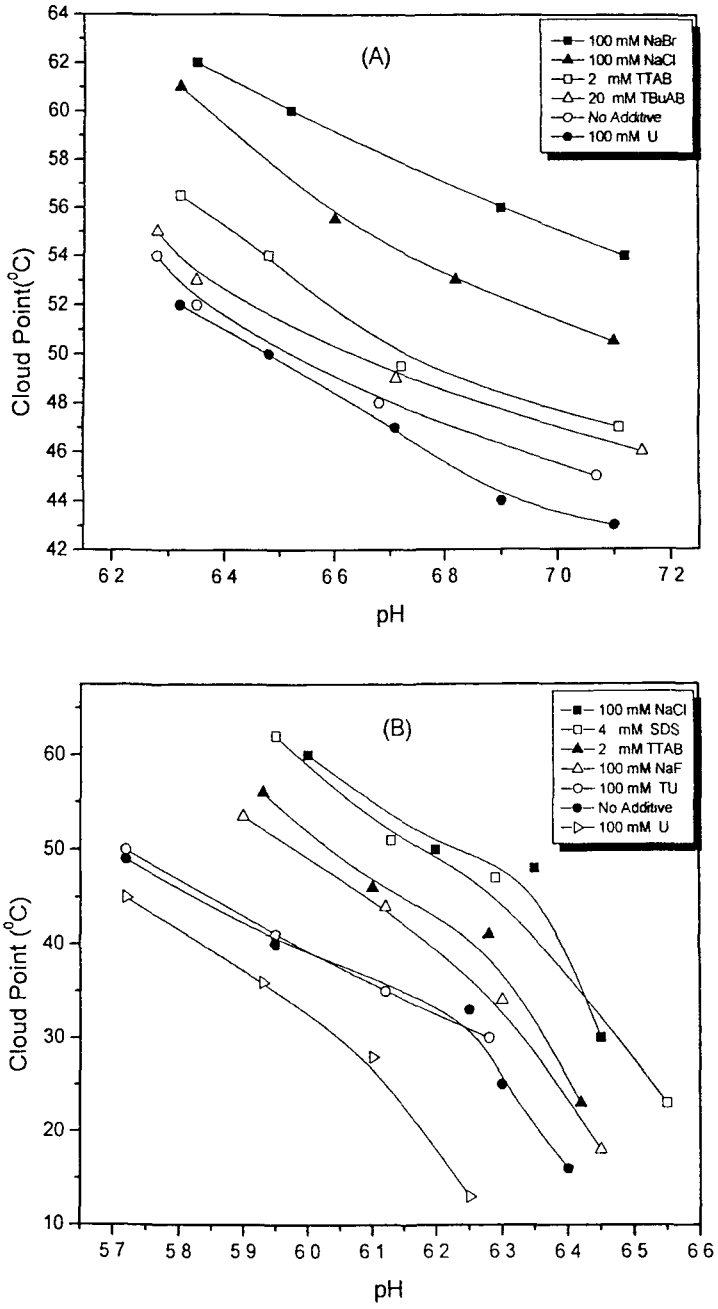

Fig. 4.1: *Effect of pH on the CP of 30 mM NOT (A) and 50 mM CLP (B) solutions, prepared in 10 mM sodium phosphate buffer, containing no or a fixed additive concentration.*

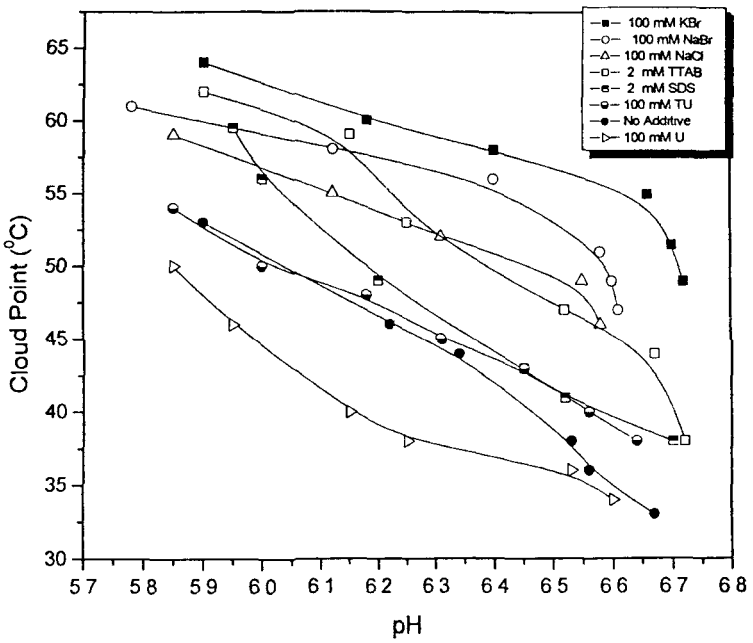

Fig. 4.2: *Effect of pH on the CP of 50 mM PMZ solutions, prepared in 10 mM sodium phosphate buffer, containing no or a fixed additive concentration.*

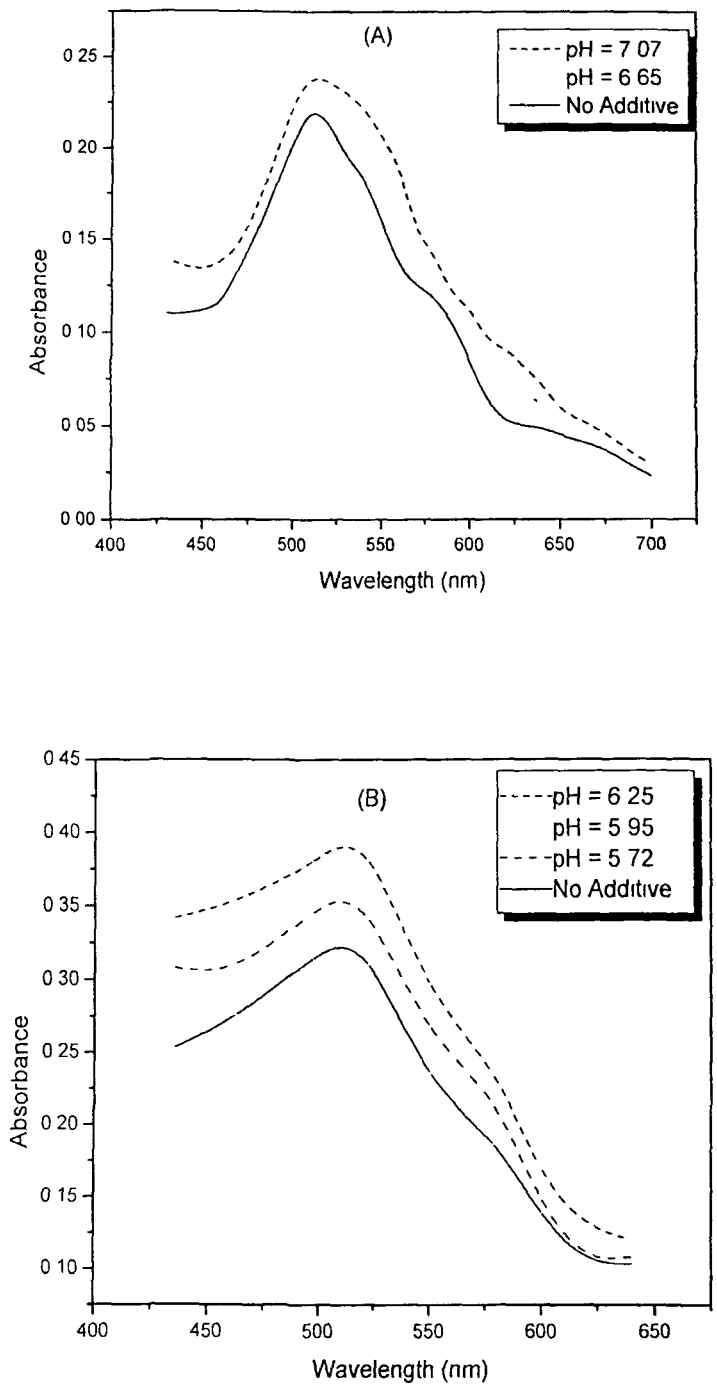

Fig. 4.3 : *Visible spectra of Sudan III in the presence of 30 mM NOT (A) and 50 mM CLP (B) in 10 mM sodium phosphate solution at different pH values.*

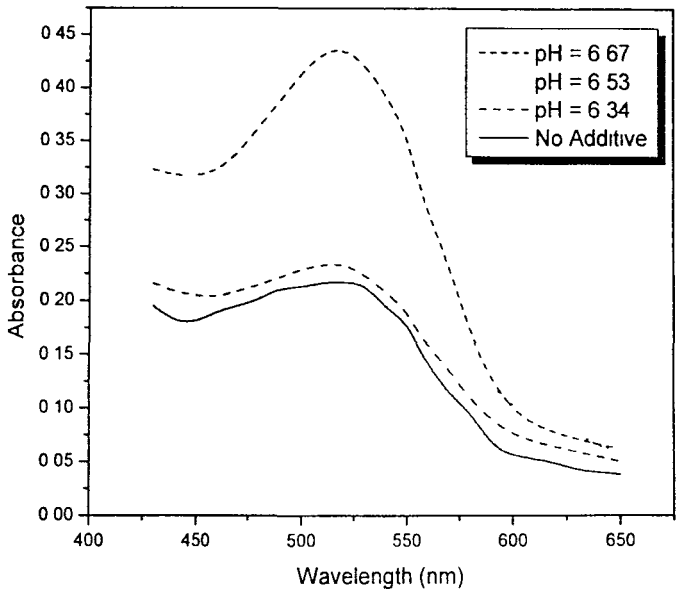

Fig. 4.4 *Visible spectra of Sudan III in the presence of 75 mM PMZ in 10 mM sodium phosphate solution at different pH values*

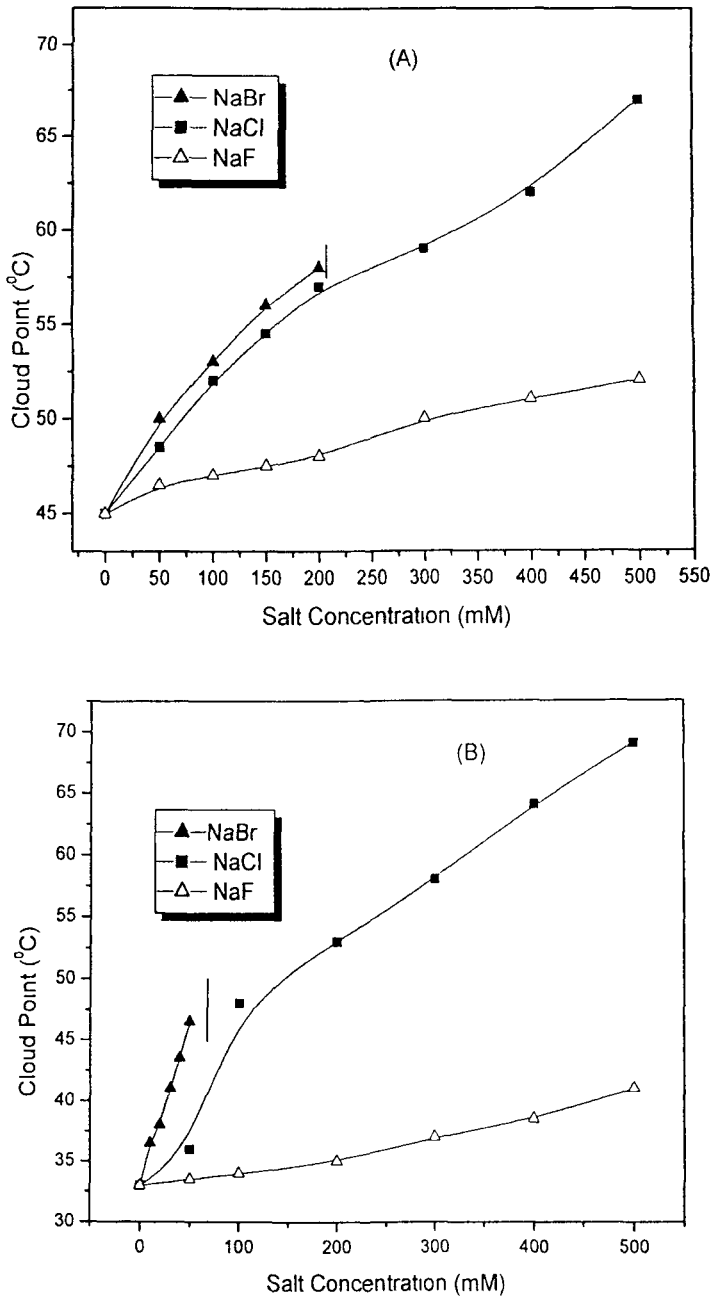

Fig. 4.5: *Effect of anionic counterions on the CP of 30 mM NOT (pH = 7.07) (A) and 50 mM CLP (pH = 6.25) (B) solutions, prepared in 10 mM sodium phosphate buffer. The vertical lines indicate precipitation occurring beyond [NaBr] at room temperature (which could be due to formation of nonmicellar phase).*

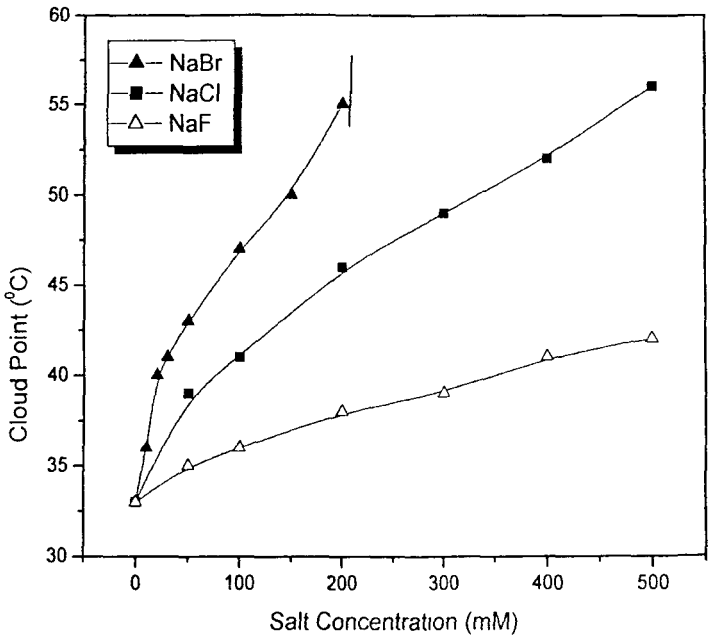

Fig. 4.6: *Effect of anionic counterions on the CP of 50 mM PMZ (pH = 6.67) solutions, prepared in 10 mM sodium phosphate buffer. The vertical line indicates precipitation occurring beyond [NaBr] at room temperature (which could be due to formation of nonmicellar phase).*

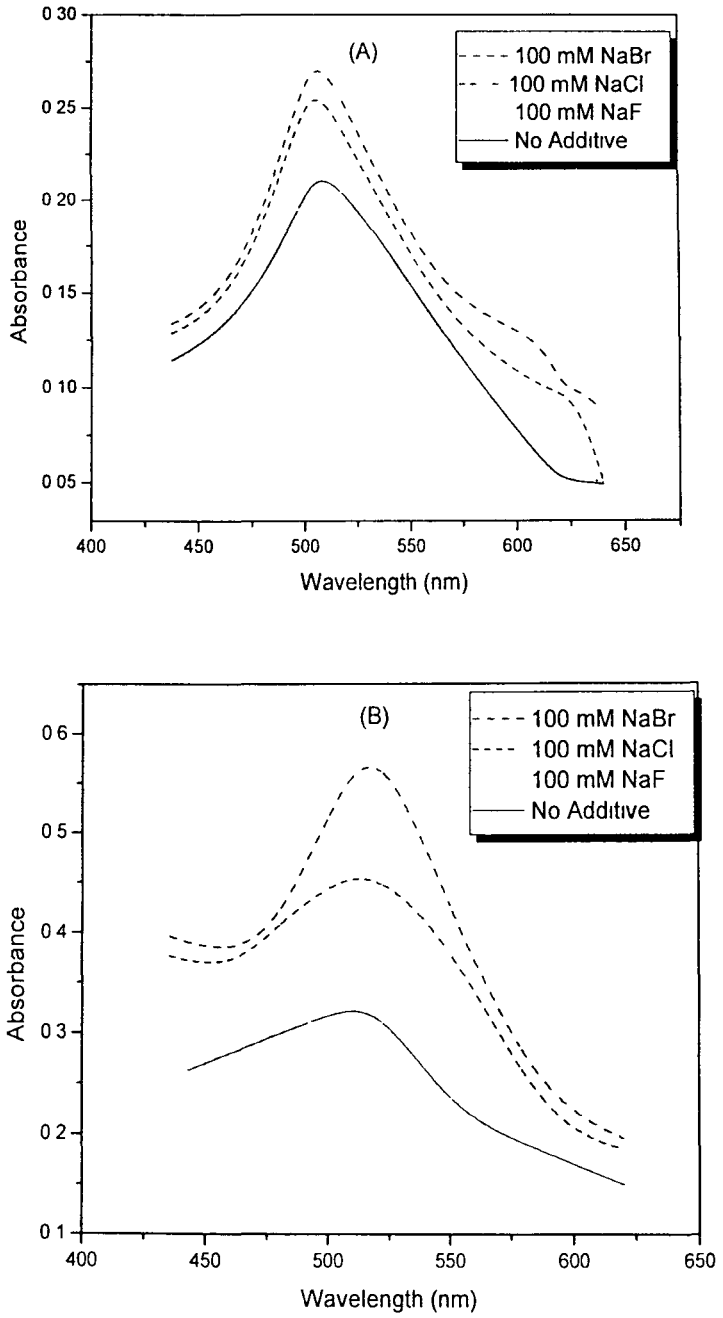

Fig. 4.7: *Visible spectra of Sudan III in the presence of 30 mM NOT (A) and 50 mM CLP (B) in water containing fixed amounts of electrolytes.*

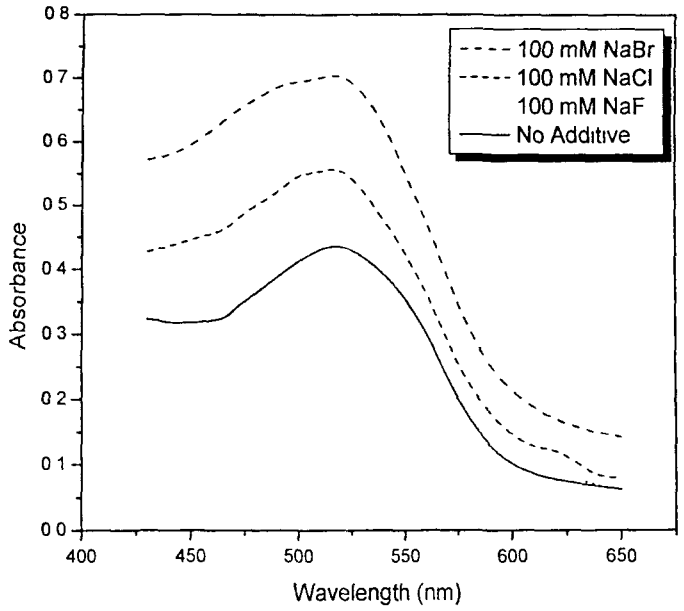

Fig. 4.8: *Visible spectra of Sudan III in the presence of 75 mM PMZ in water containing fixed amounts of electrolytes*

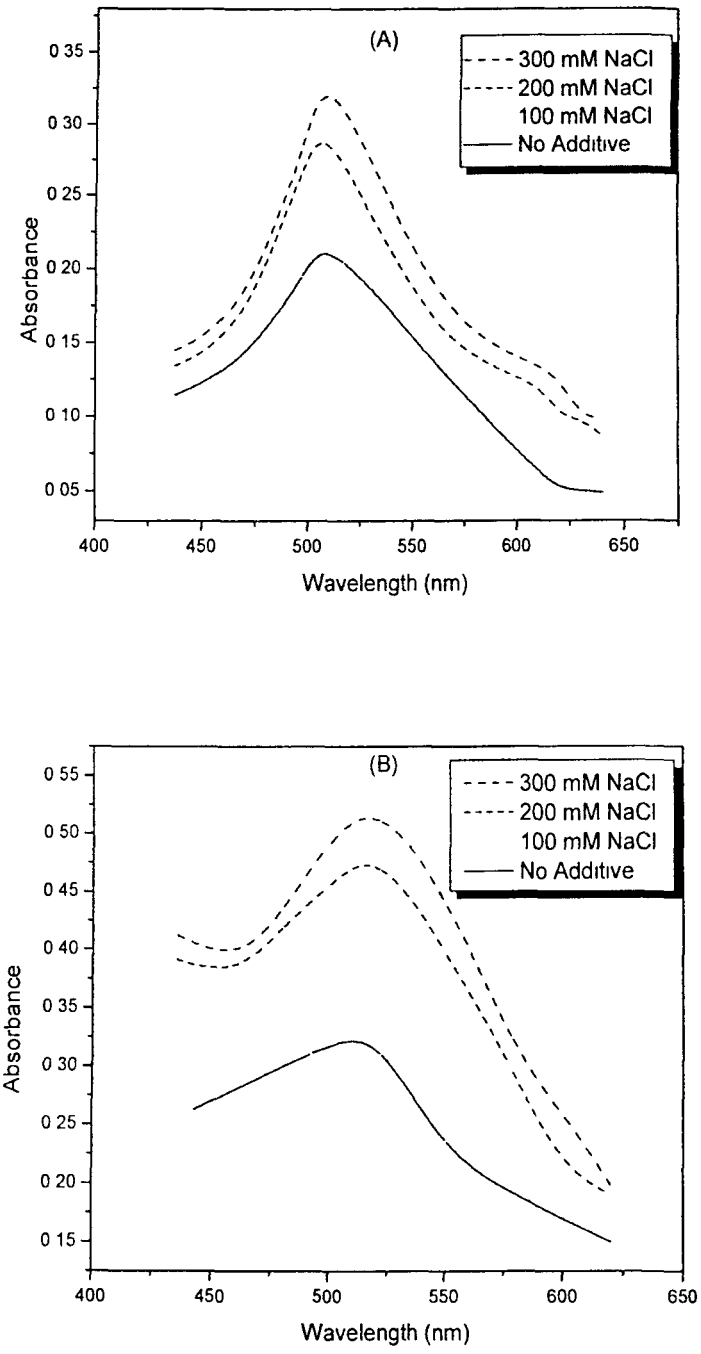

Fig. 4.9: *Visible spectra of Sudan III in the presence of 30 mM NOT (A) and 50 mM CLP (B) in water containing various fixed amounts of NaCl.*

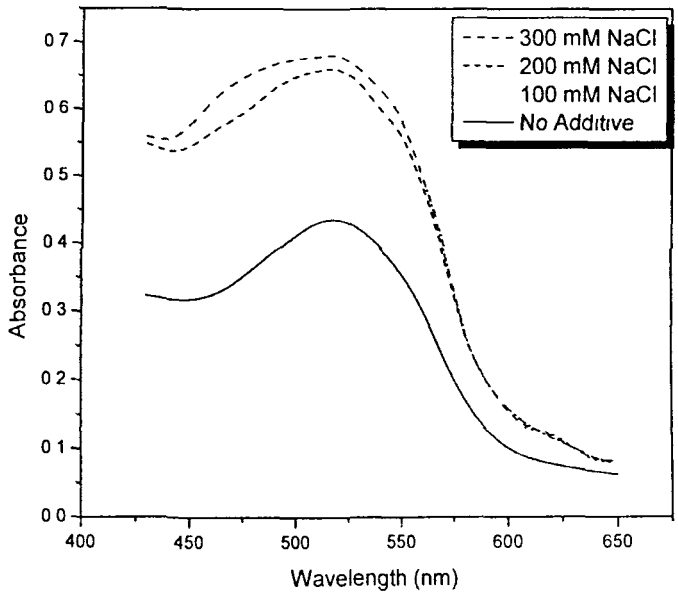

Fig. 4.10: *Visible spectra of Sudan III in the presence of 75 mM PMZ in water containing various fixed amounts of NaCl*

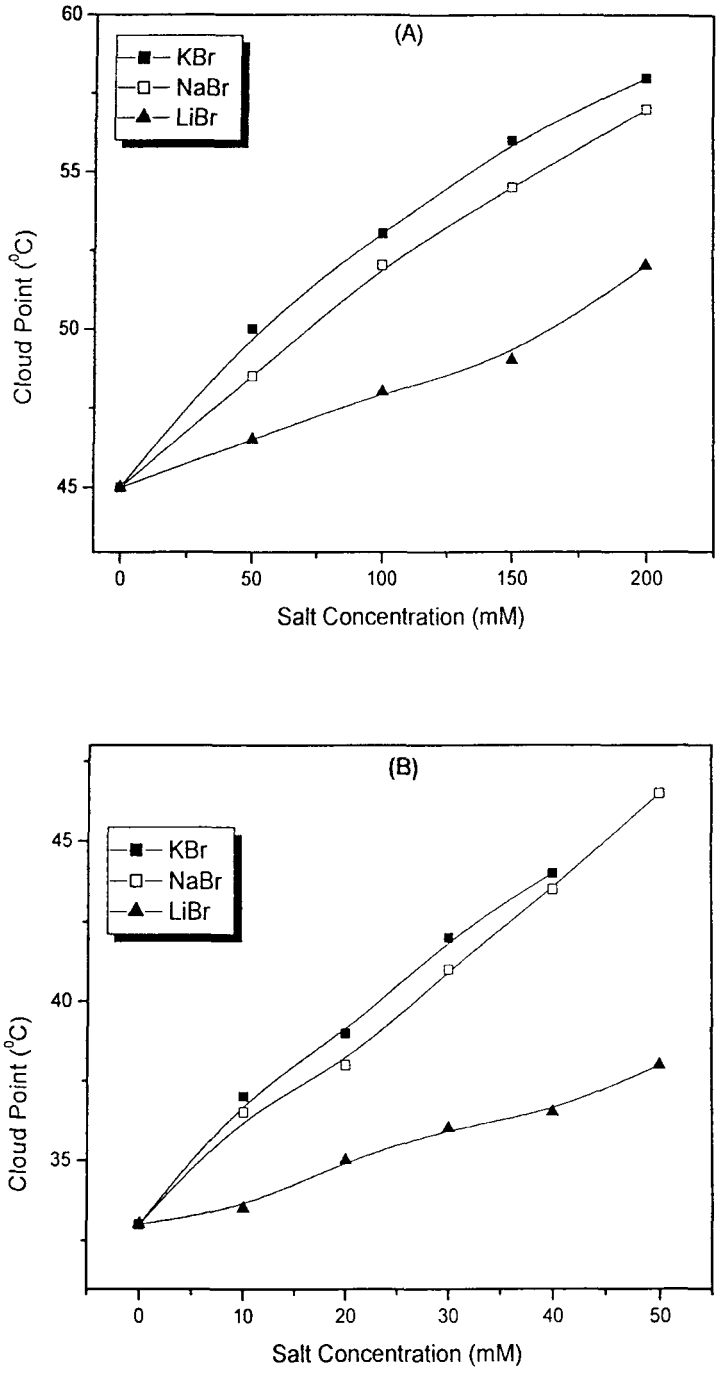

Fig. 4.11: *Effect of cationic coions on the CP of 30 mM NOT (pH =7.07) (A) and 50 mM CLP (pH=6.25) (B) solutions, prepared in 10 mM sodium phosphate buffer.*

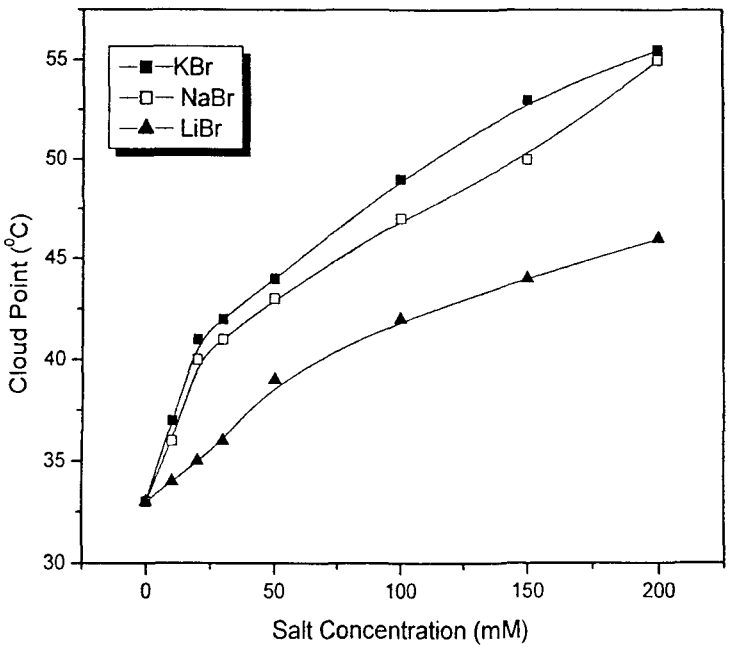

Fig. 4.12: *Effect of cationic coions on the CP of 50 mM PMZ (pH =6.67) solutions, prepared in 10 mM sodium phosphate buffer.*

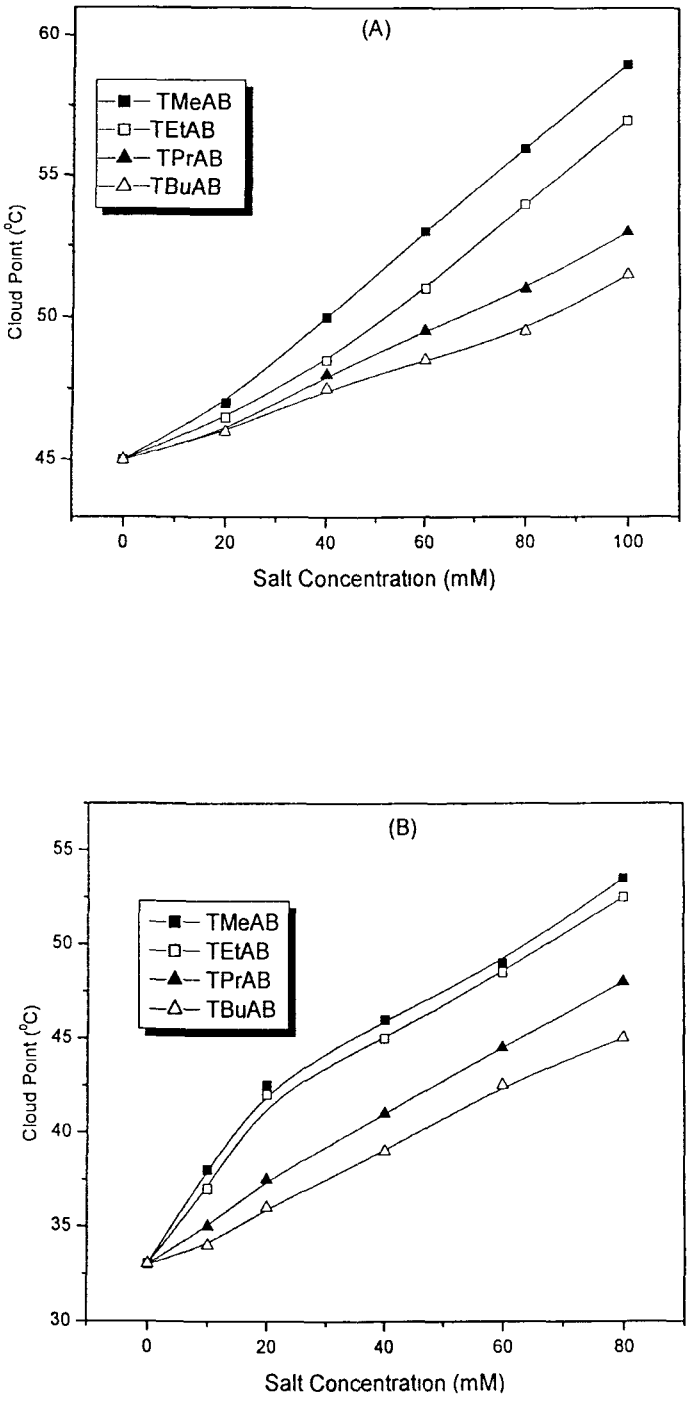

Fig. 4.13: *Effect of quaternary ammonium bromide salt concentration on the CP of 30 mM NOT (pH = 7.07) (A) and 50 mM CLP (pH= 6.25) (B) solutions, prepared in 10 mM sodium phosphate buffer.*

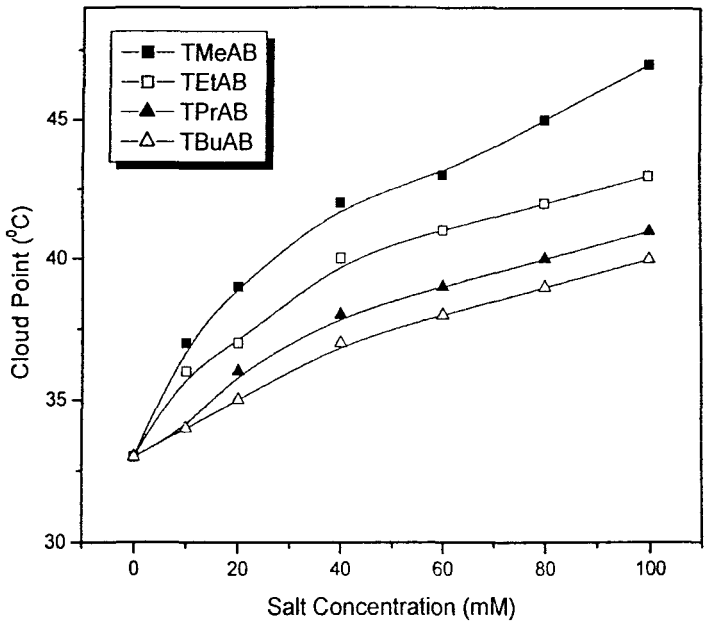

Fig. 4.14: *Effect of quaternary ammonium bromide salt concentration on the CP of 50 mM PMZ (pH = 6.67) solutions, prepared in 10 mM sodium phosphate buffer.*

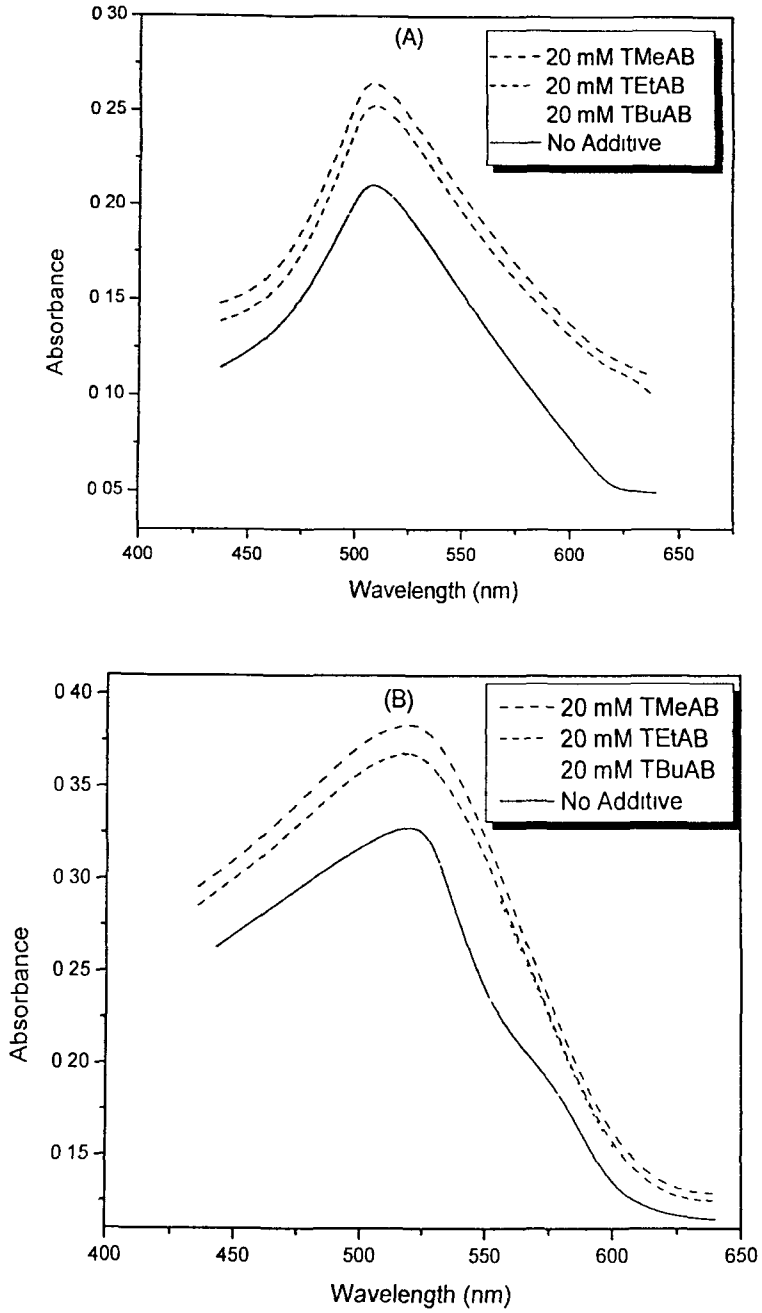

Fig. 4.15: *Visible spectra of Sudan III in the presence of 30 mM NOT (A) and 50 mM CLP(B) in water containing fixed amounts of quaternary ammonium salts*

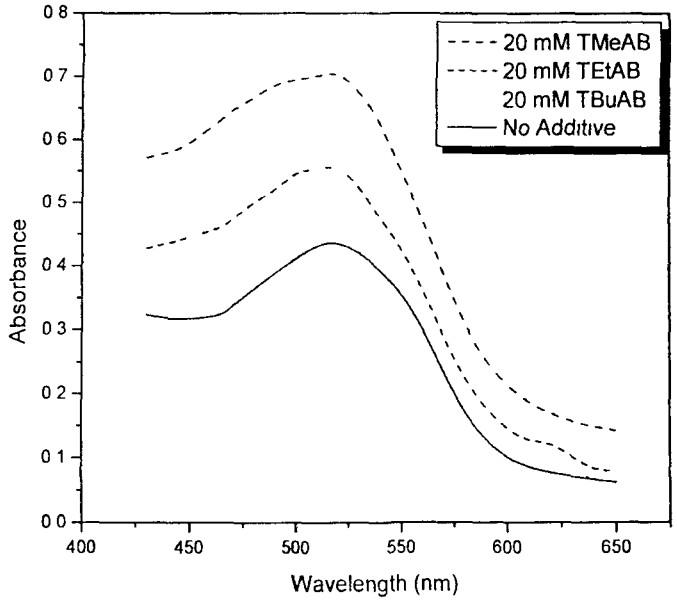

Fig. 4.16· *Visible spectra of Sudan III in the presence of 75 mM PMZ in water containing fixed amounts of quaternary ammonium salts.*

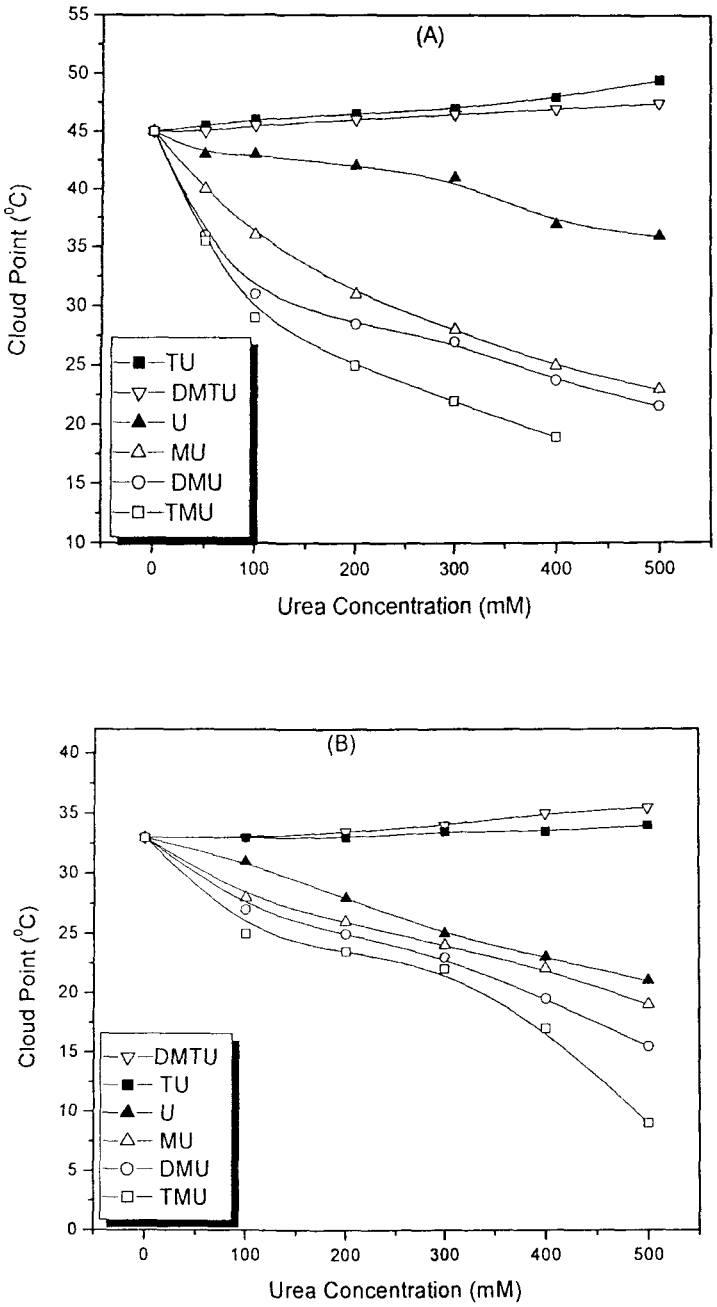

Fig. 4.17: *Effect of ureas concentration on the CP of 30 mM NOT (pH = 7.07) (A) and 50 mM CLP (pH= 6.25) (B) solutions, prepared in 10 mM sodium phosphate buffer.*

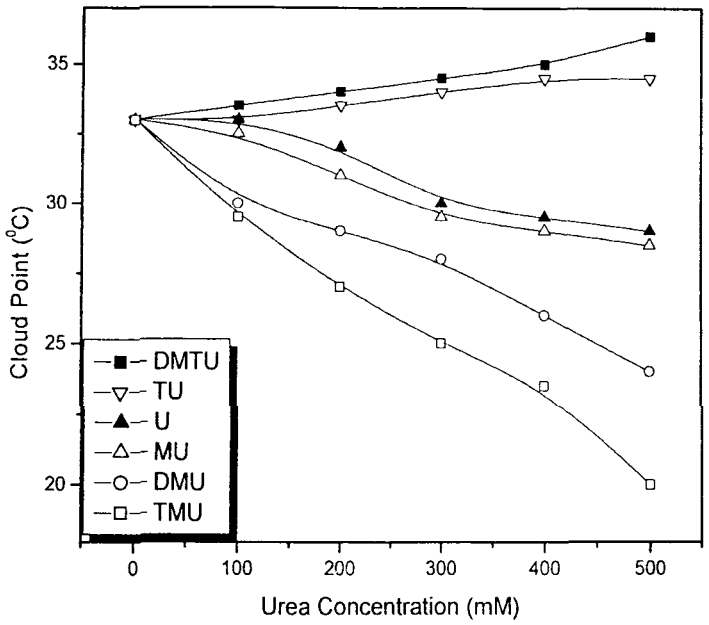

Fig. 4.18: *Effect of ureas concentration on the CP of 50 mM PMZ (pH = 6.67) solutions, prepared in 10 mM sodium phosphate buffer.*

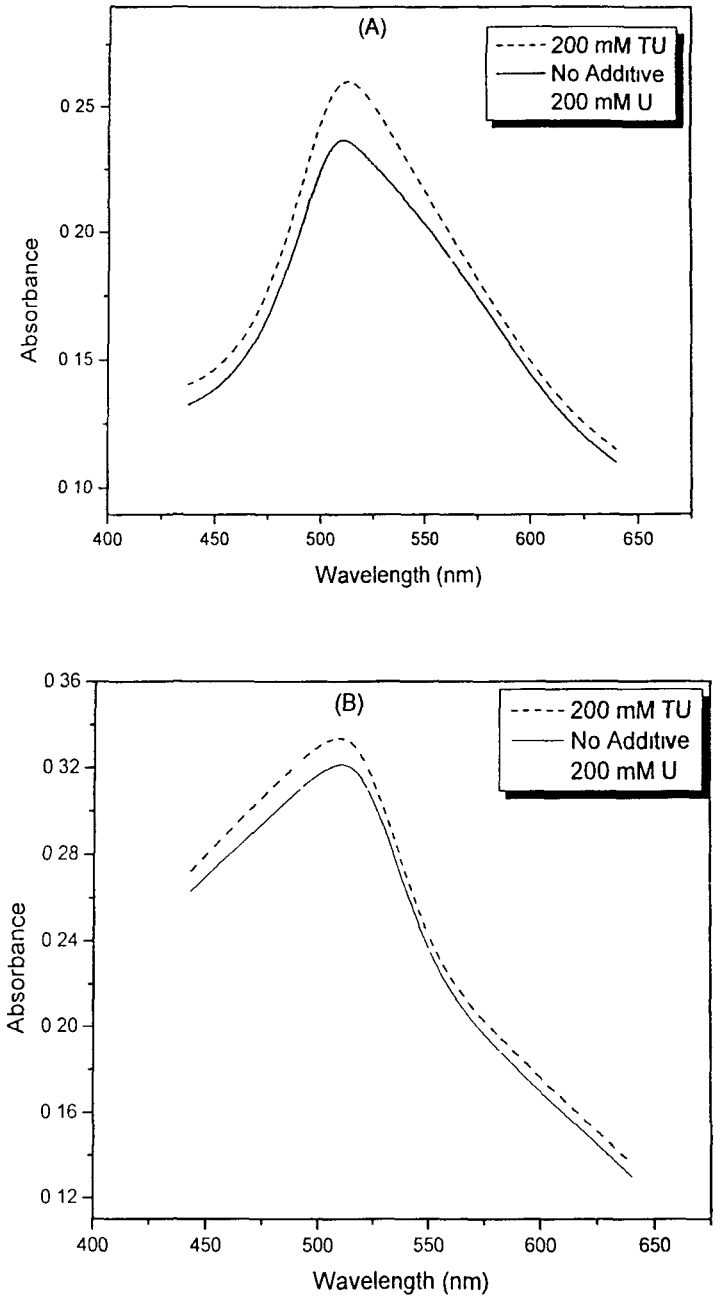

Fig. 4.19: *Visible spectra of Sudan III in the presence of 30 mM NOT (A) and 50 mM CLP(B) in water containing urea and thiourea*

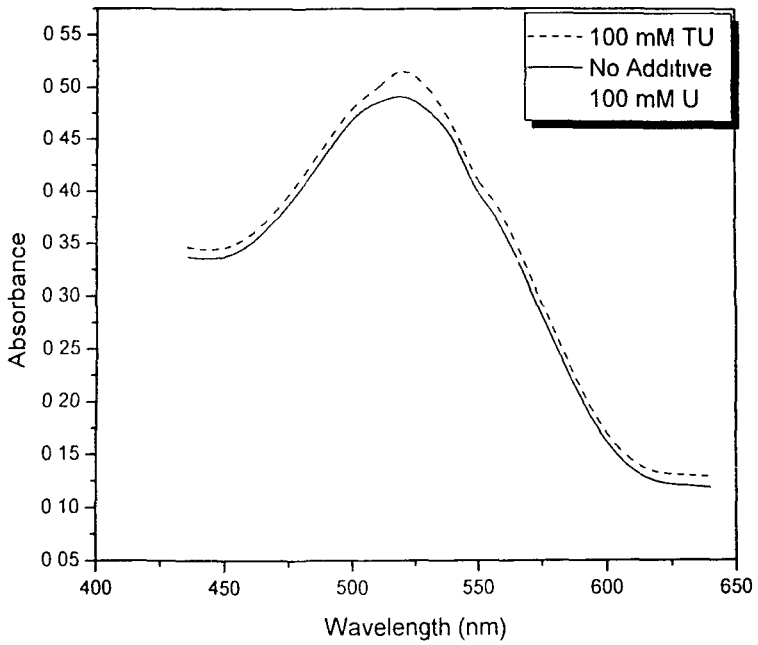

Fig. 4.20: *Visible spectra of Sudan III in the presence of 75 mM PMZ in water containing urea and thiourea*

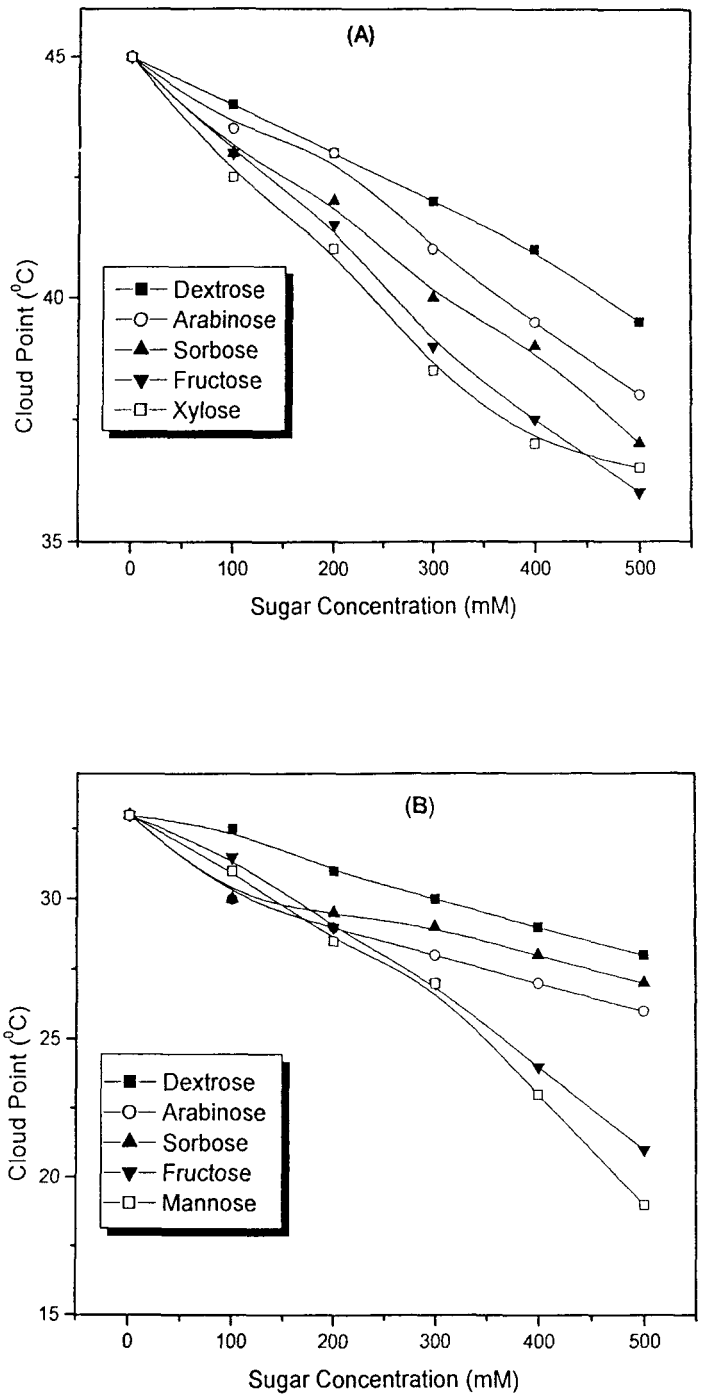

Fig. 4.21: *Effect of sugar concentration on the CP of 30 mM NOT (pH = 7.07) (A) and 50 mM CLP (pH= 6.25) (B) solutions, prepared in 10 mM sodium phosphate buffer.*

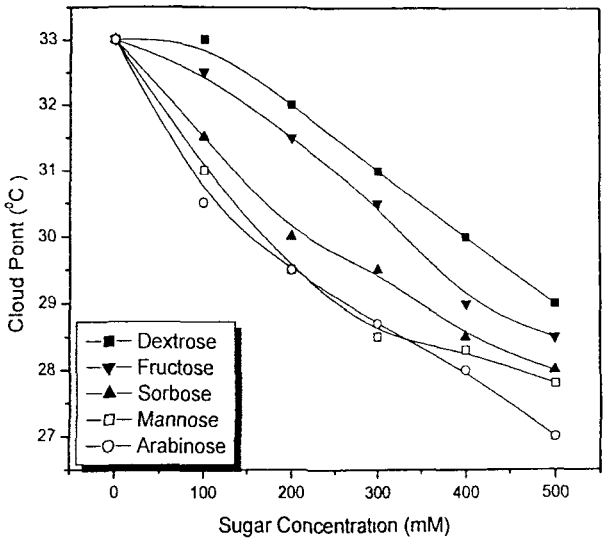

Fig. 4.22: *Effect of sugar concentration on the CP of 50 mM PMZ (pH = 6.67) solutions, prepared in 10 mM sodium phosphate buffer.*

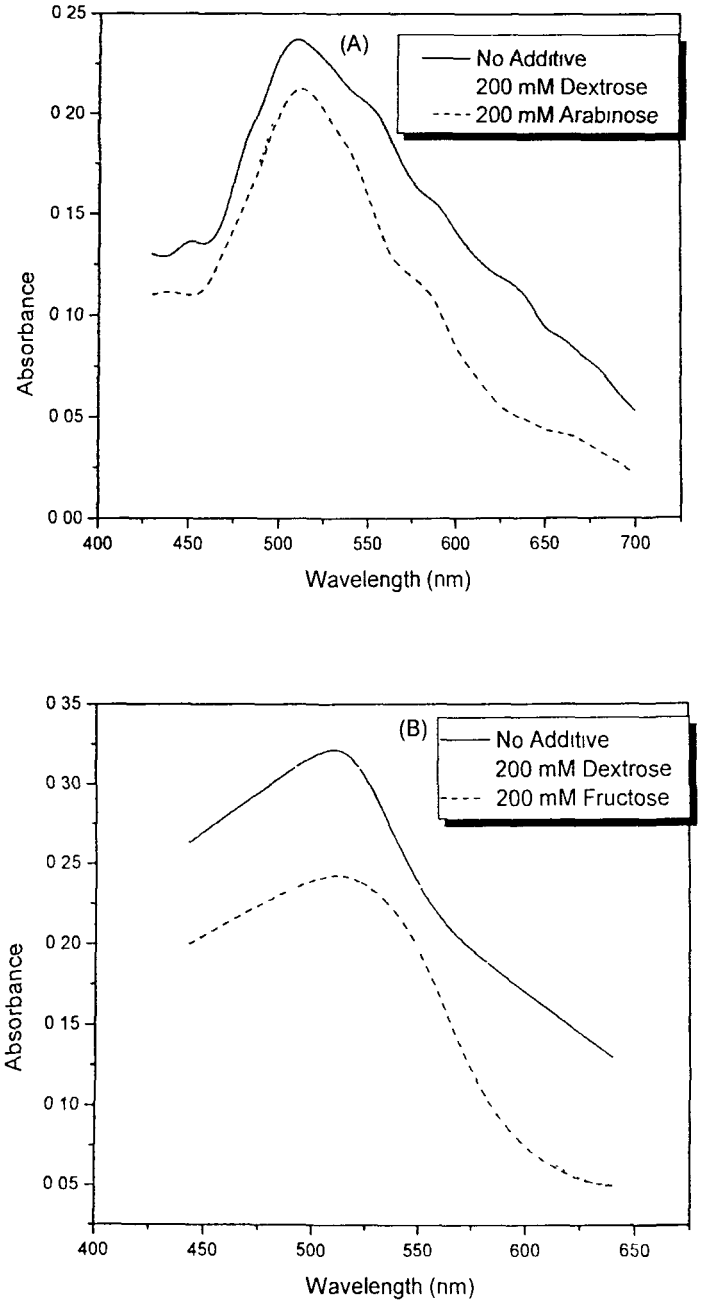

Fig. 4.23: *Visible spectra of Sudan III in the presence of 45 mM NOT (A) and 50 mM CLP(B) in water containing different sugars.*

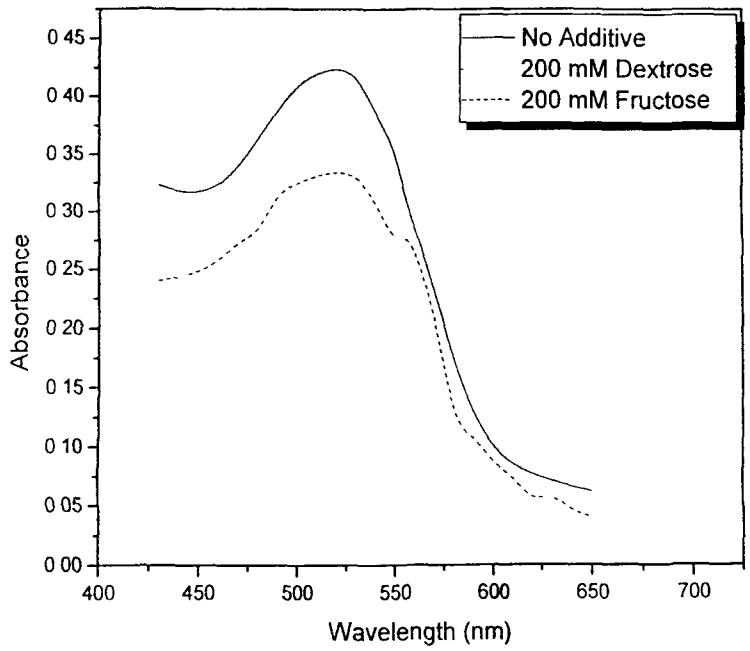

Fig. 4.24: *Visible spectra of Sudan III in the presence of 75 mM PMZ in water containing different sugars.*

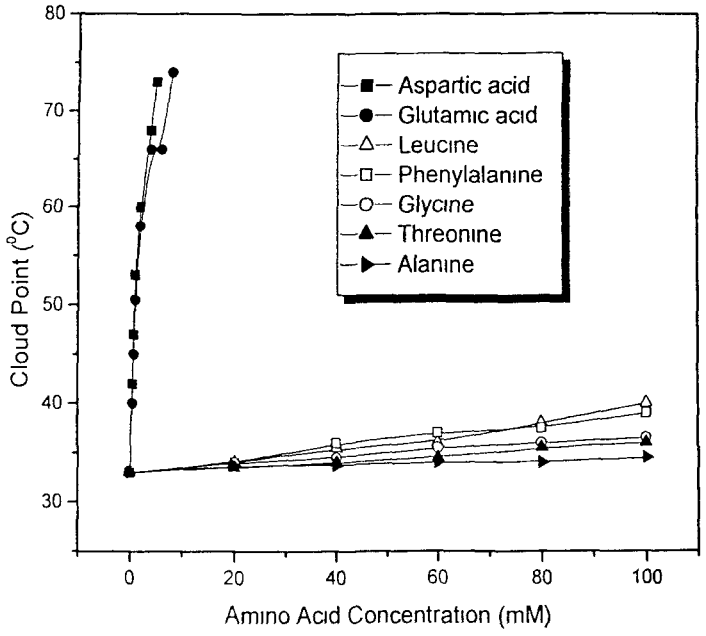

Fig. 4.25: *Effect of amino acid concentration on the CP of 50 mM CLP (pH = 6.25) solutions, prepared in 10 mM sodium phosphate buffer.*

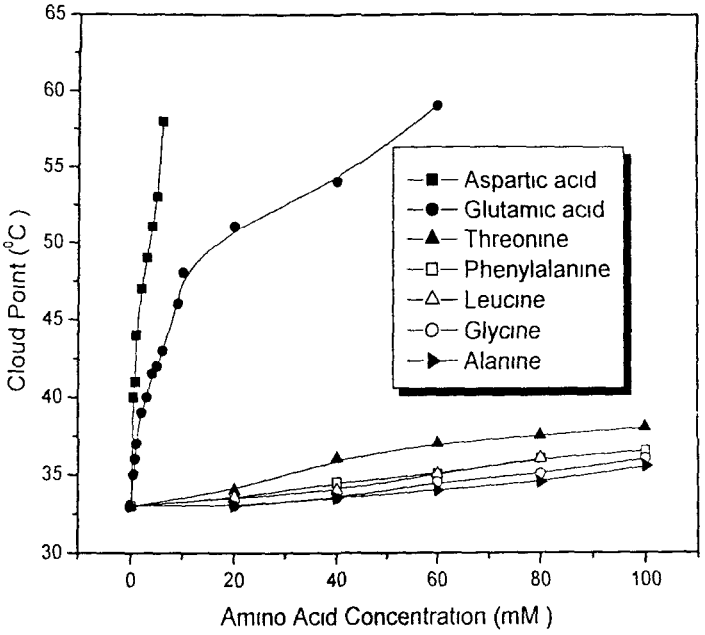

Fig. 4.26: *Effect of amino acid concentration on the CP of 50 mM PMZ (pH = 6 67) solutions, prepared in 10 mM sodium phosphate buffer*

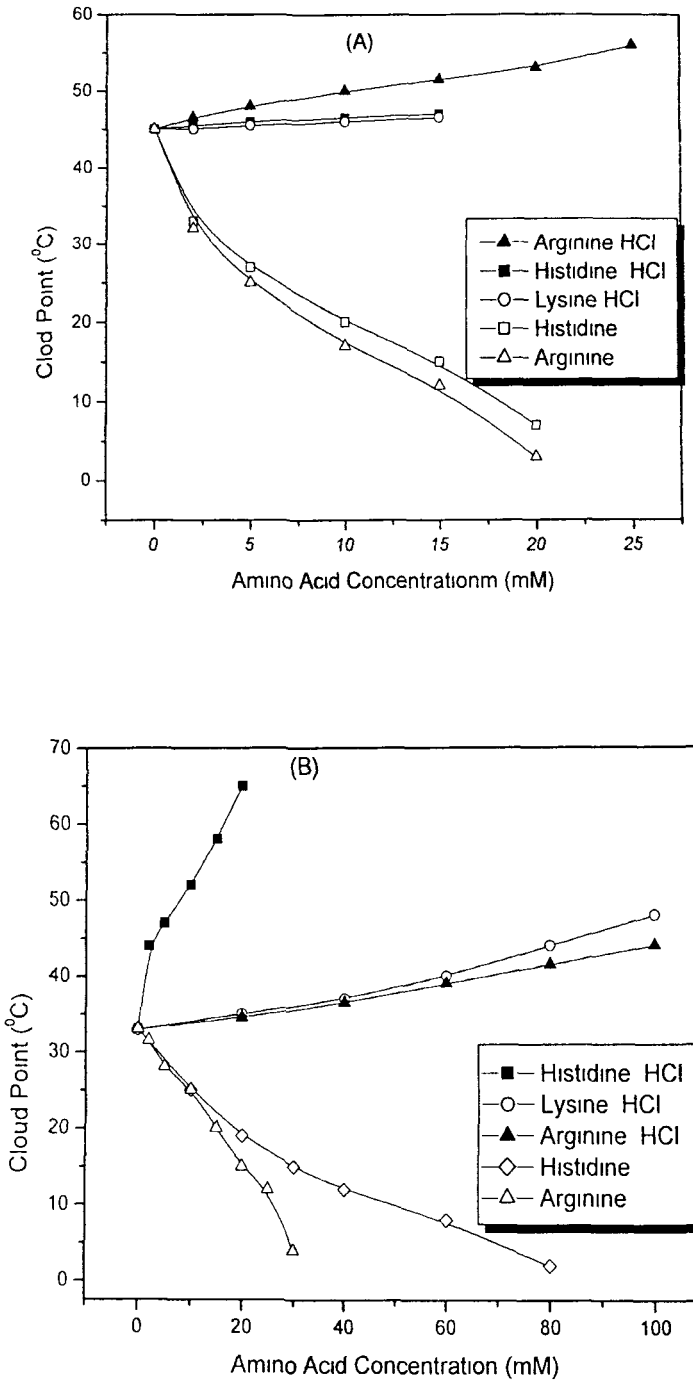

Fig. 4.27: *Effect of amino acid concentration on the CP of 30 mM NOT (pH = 7.07) (A) and 50 mM CLP (pH= 6.25) (B) solutions, prepared in 10 mM sodium phosphate buffer.*

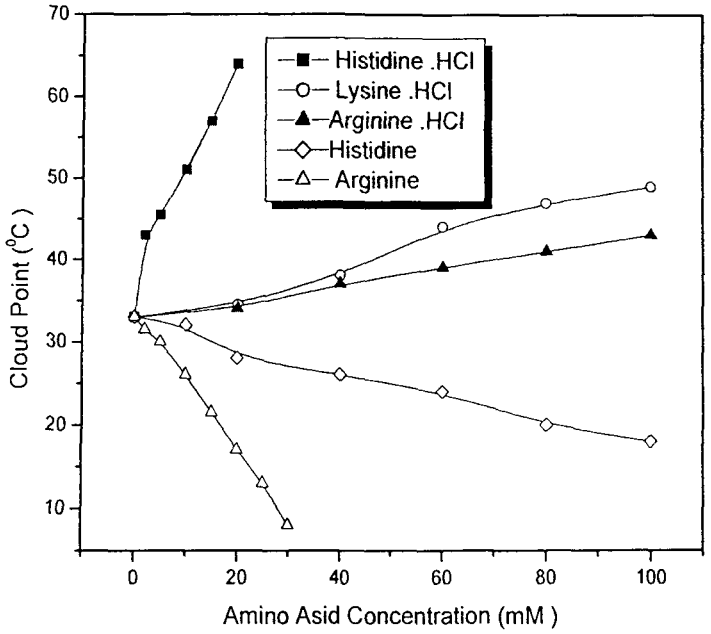

Fig. 4.28: *Effect of amino acid concentration on the CP of 50 mM PMZ (pH = 6.67) solutions, prepared in 10 mM sodium phosphate buffer.*

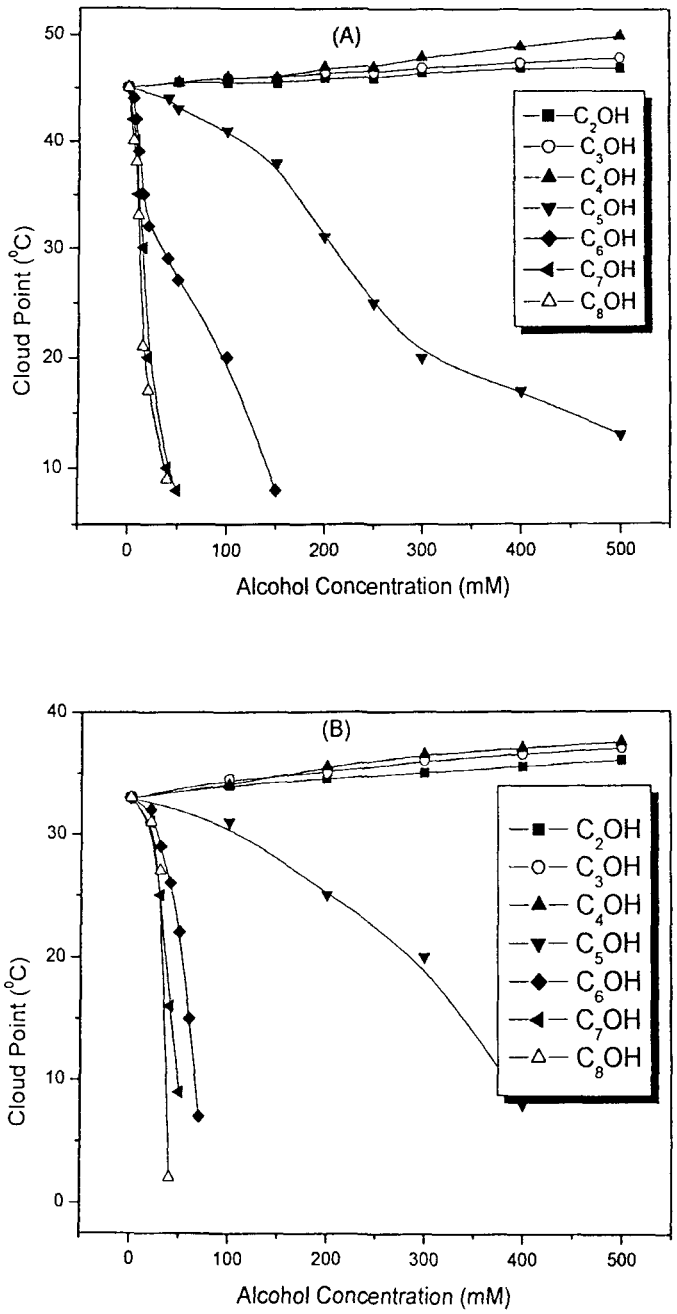

Fig. 4.29: *Effect of alcohol concentration on the CP of 30 mM NOT (pH = 7.07) (A) and 50 mM CLP (pH= 6.25) (B) solutions, prepared in 10 mM sodium phosphate buffer.*

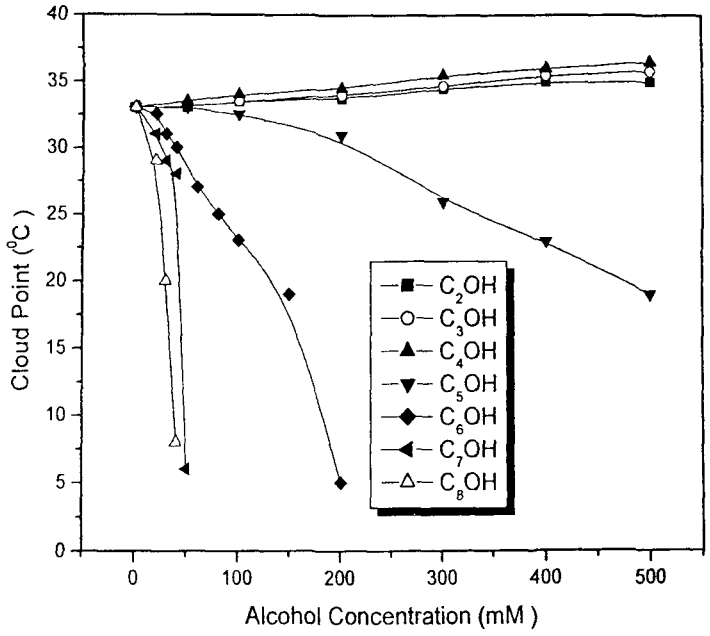

Fig. 4.30: *Effect of alcohol concentration on the CP of 50 mM PMZ (pH = 6.67) solutions, prepared in 10 mM sodium phosphate buffer.*

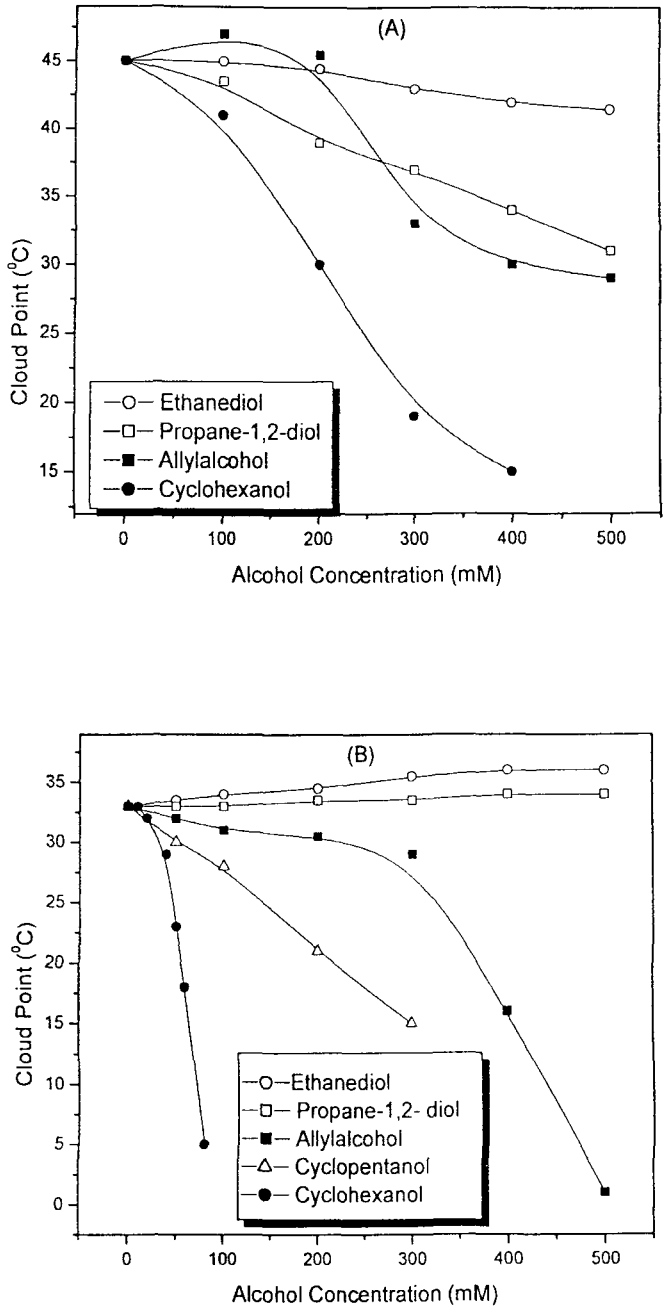

Fig. 4.31: *Effect of alcohol concentration on the CP of 30 mM NOT (pH = 7.07) (A) and 50 mM CLP (pH= 6.25) (B) solutions, prepared in 10 mM sodium phosphate buffer.*

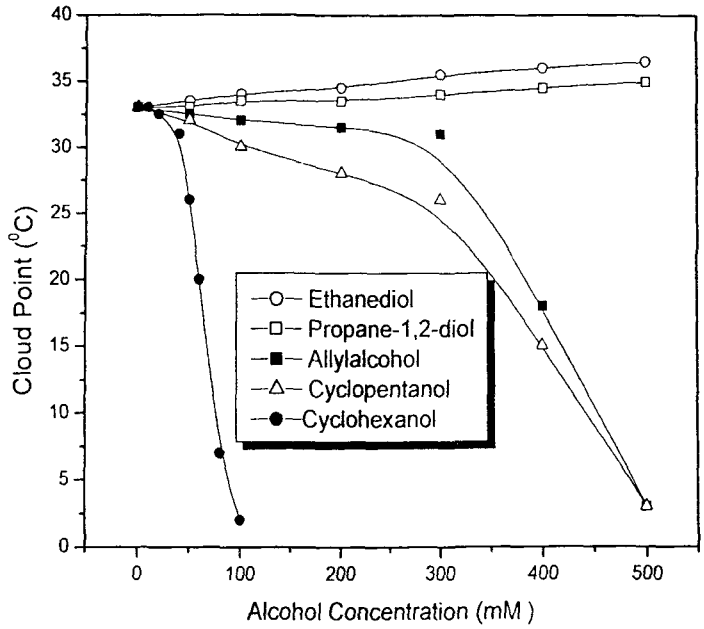

Fig. 4.32: *Effect of alcohol concentration on the CP of 50 mM PMZ (pH = 6.67) solutions, prepared in 10 mM sodium phosphate buffer.*

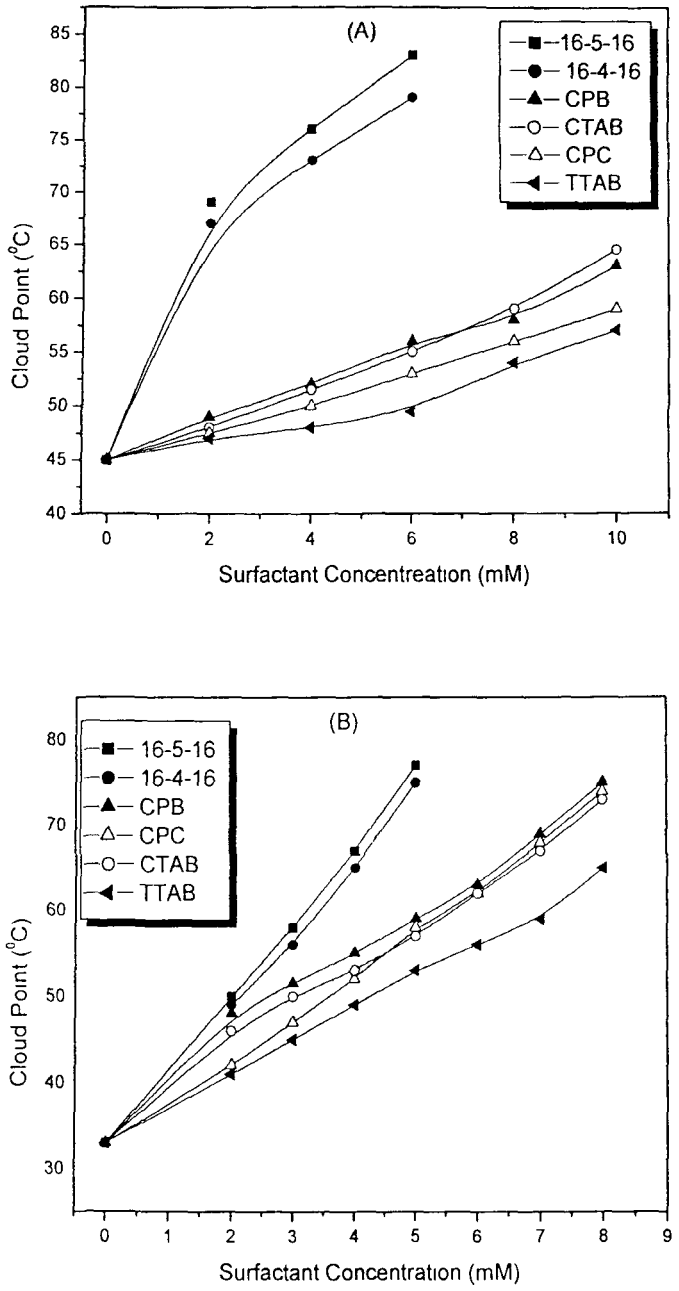

Fig. 4.33: *Effect of cationic surfactant concentration on the CP of 30 mM NOT (pH 7.07) (A) and 50 mM CLP (pH= 6.25) (B) solutions, prepared in 10 mM sodium phosphate buffer.*

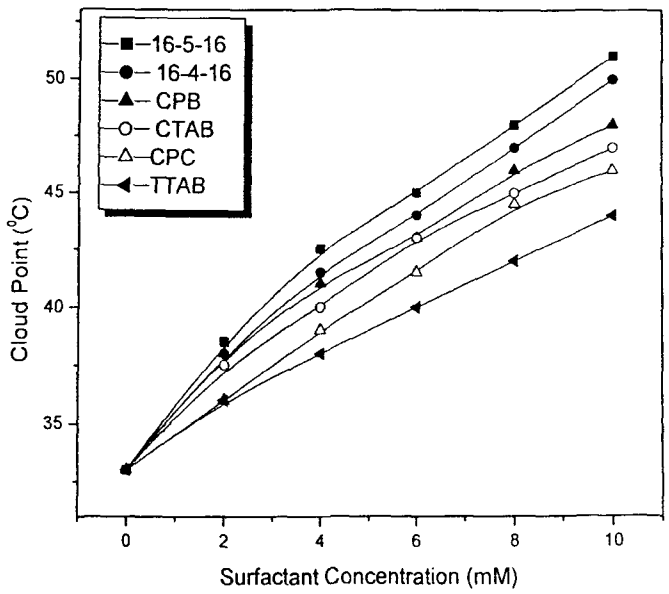

Fig. 4.34: *Effect of cationic surfactant concentration on the CP of 50 mM PMZ (pH = 6.67) solutions, prepared in 10 mM sodium phosphate buffer.*

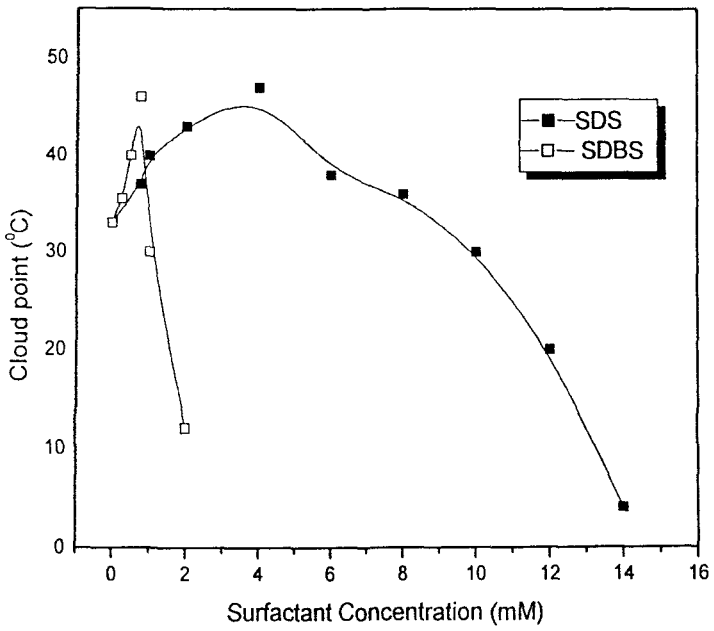

Fig. 4.35: *Effect of anionic surfactant concentration on the CP of 50 mM CLP (pH = 6.25) solutions, prepared in 10 mM sodium phosphate buffer.*

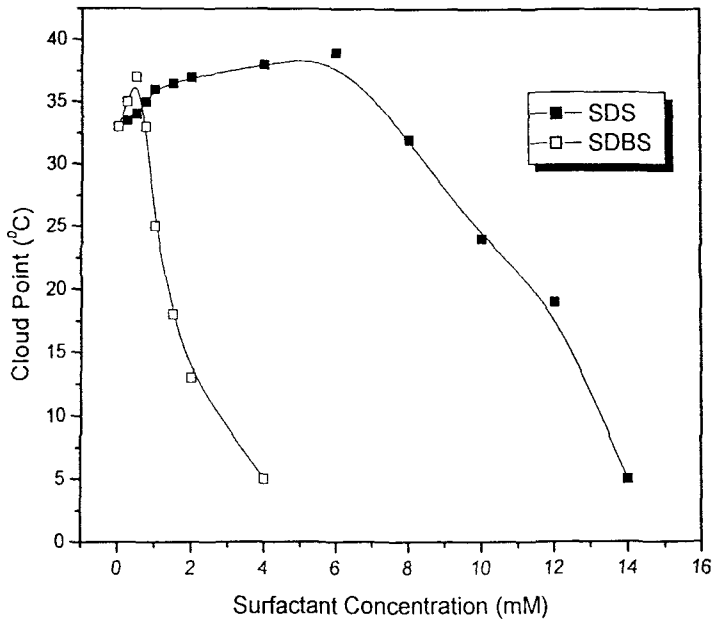

Fig. 4.36: *Effect of anionic surfactant concentration on the CP of 50 mM PMZ (pH = 6.67) solutions, prepared in 10 mM sodium phosphate buffer.*

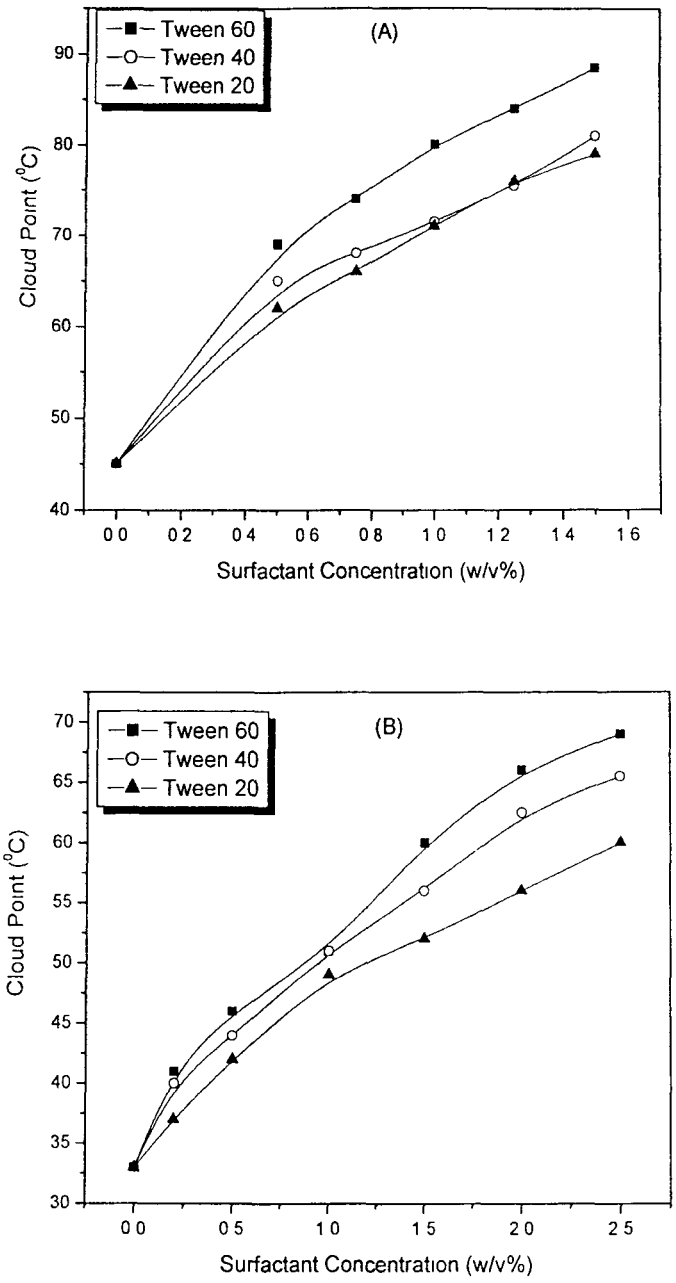

Fig. 4.37: *Effect of non-ionic surfactant concentration on the CP of 30 mM NOT (pH = 7.07) (A) and 50 mM CLP (pH= 6.25) (B) solutions, prepared in 10 mM sodium phosphate buffer.*

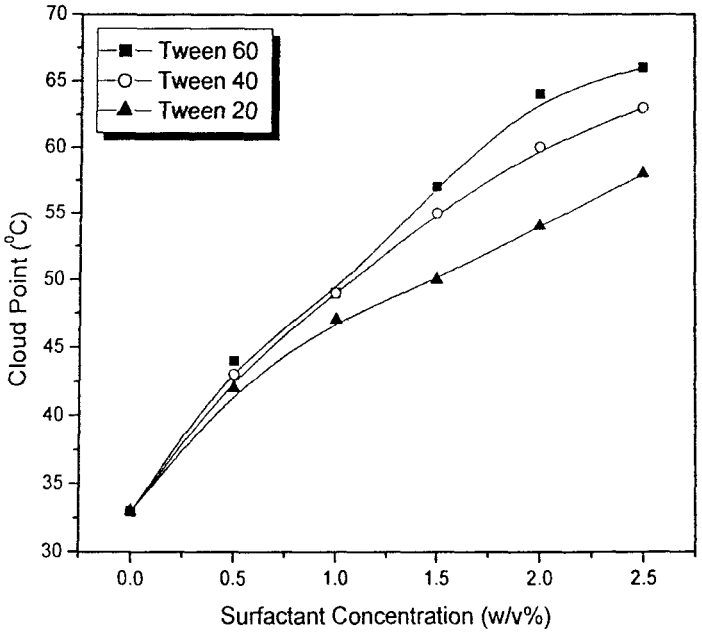

Fig. 4.38: *Effect of non-ionic surfactant concentration on the CP of 50 mM PMZ (pH = 6.67) solutions, prepared in 10 mM sodium phosphate buffer.*

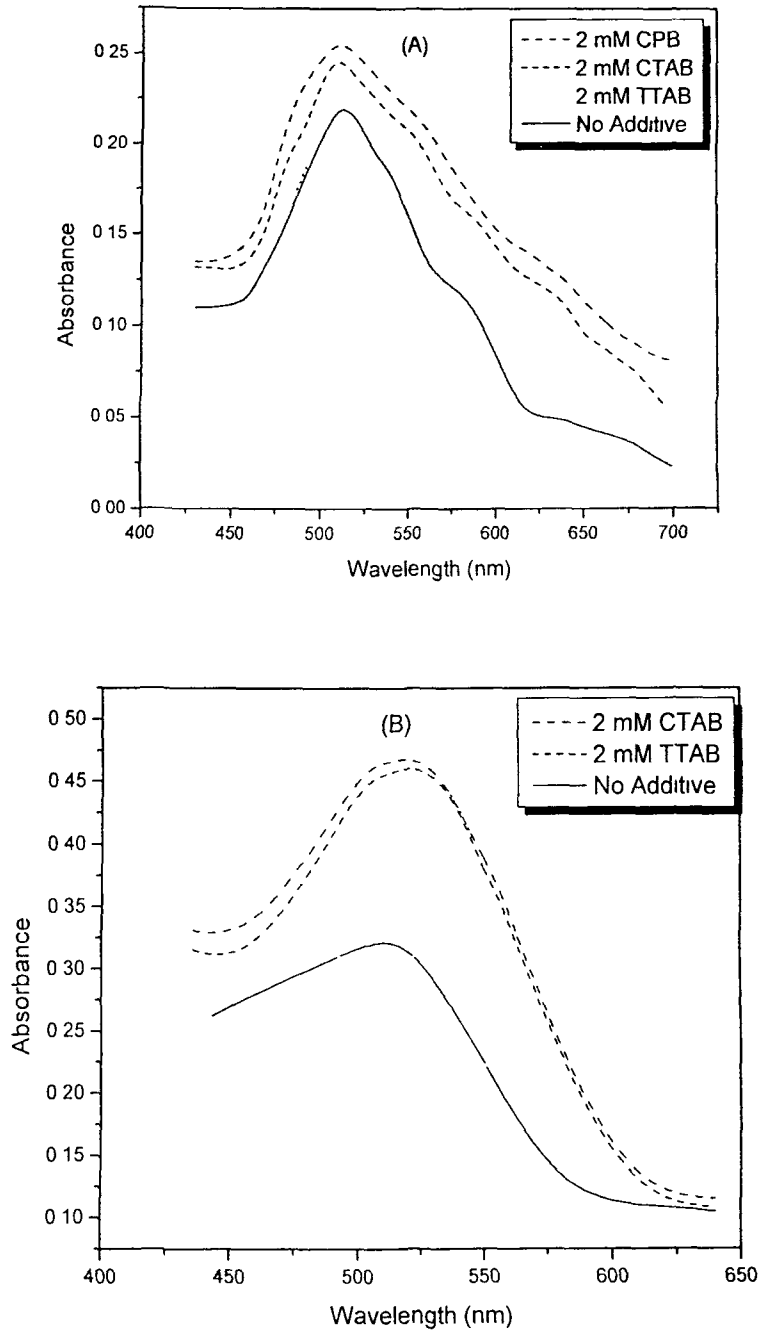

Fig. 4.39: *Visible spectra of Sudan III in the presence of 30 mM NOT(A) and 50 mM CLP(B) in water containing fixed amounts of surfactants .*

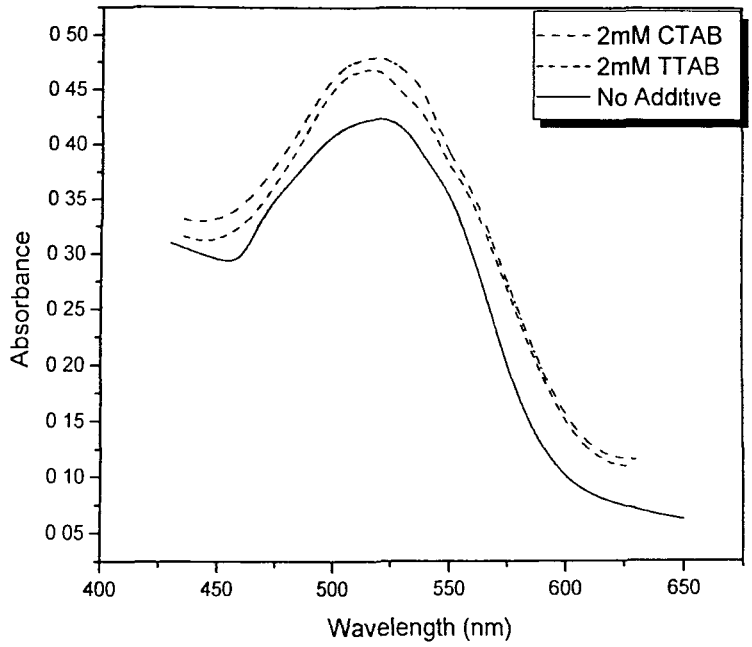

Fig.4.40: *Visible spectra of Sudan III in the presence of 75 mM PMZ in water containing fixed amounts of surfactants.*

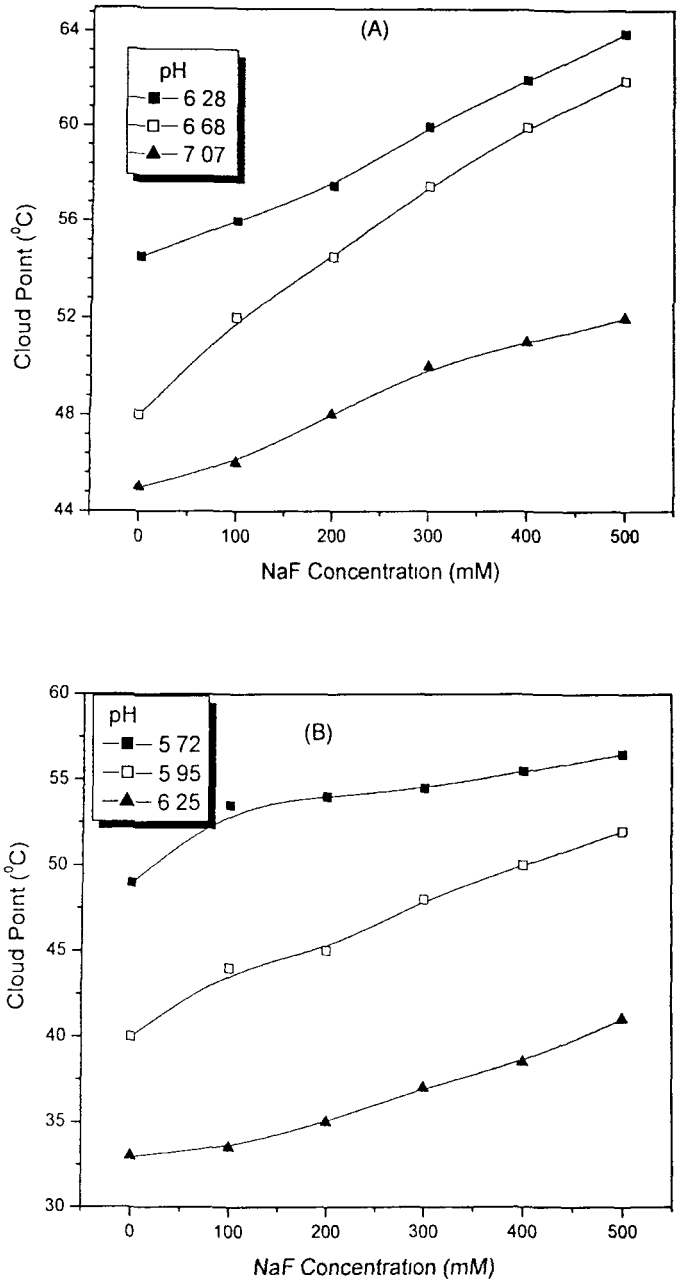

Fig. 4.41: *Effect of NaF concentration on the CP of 30 mM NOT (A) and 50 mM CLP (B) solutions, prepared in 10 mM sodium phosphate buffer at different pH's.*

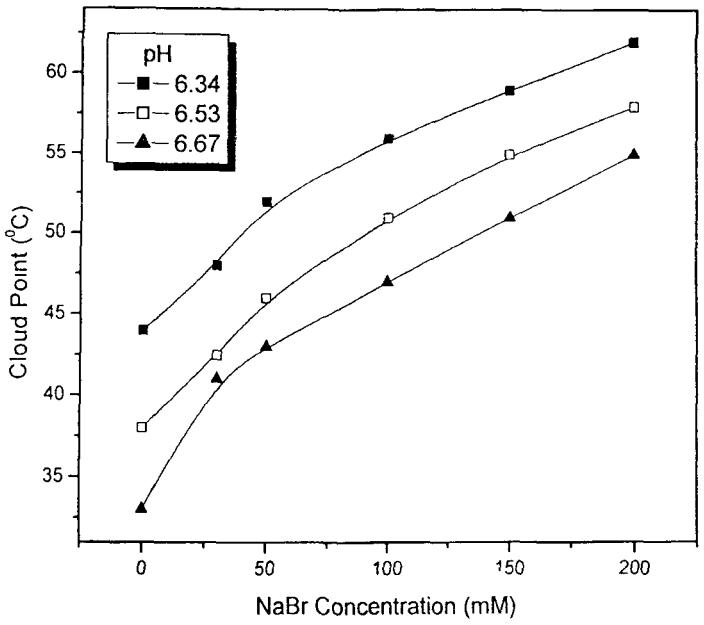

Fig. 4.42: *Effect of NaBr concentration on the CP of 50 mM PMZ solutions, prepared in 10 mM sodium phosphate buffer at different pH's.*

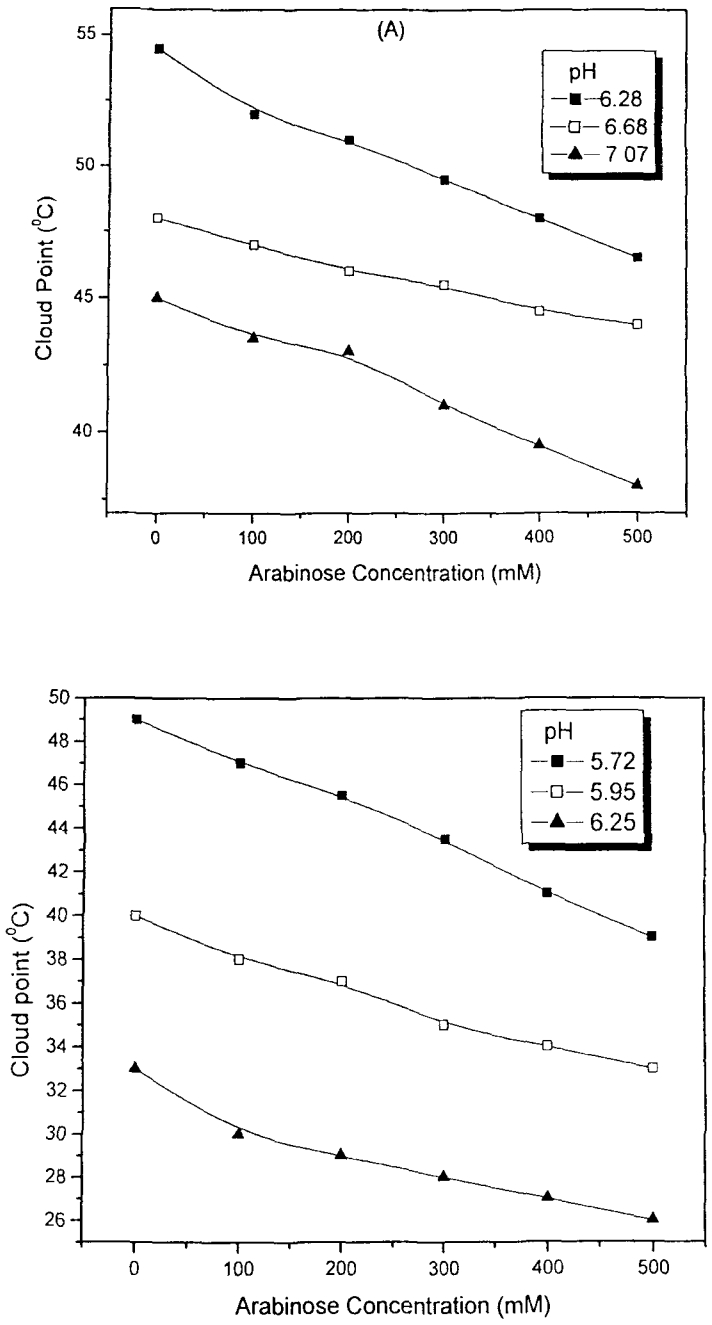

Fig. 4.43: *Effect of arabinose concentration on the CP of 30 mM NOT (A) and 50 mM CLP (B) solutions, prepared in 10 mM sodium phosphate buffer at different pH's.*

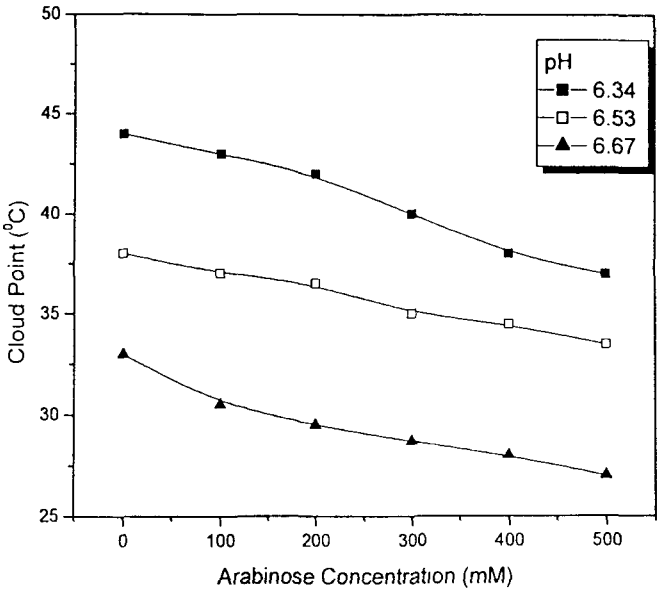

Fig. 4.44: *Effect of arabinose concentration on the CP of 50 mM PMZ solutions, prepared in 10 mM sodium phosphate buffer at different pH's.*

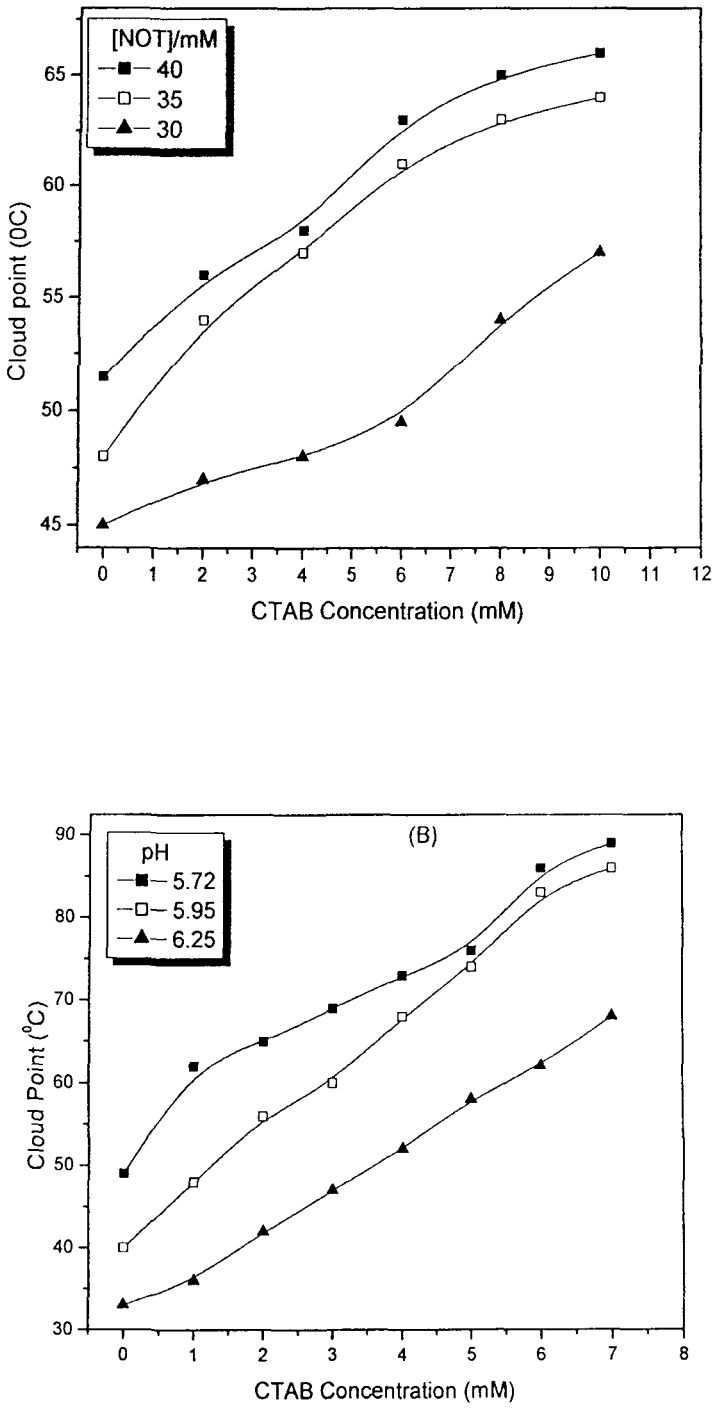

Fig. 4.45: *Effect of CTAB concentration on the CP of 30 mM NOT (A) and 50 mM CLP (B) solutions, prepared in 10 mM sodium phosphate buffer at different pH's.*

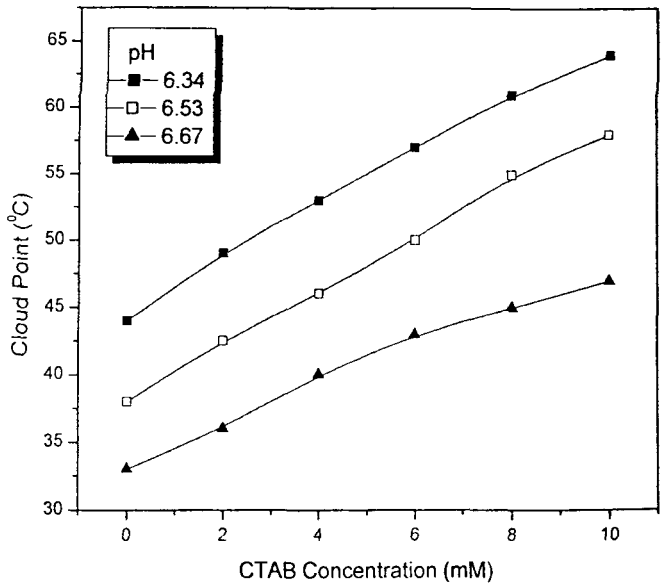

Fig. 4.46: *Effect of CTAB concentration on the CP of 50 mM PMZ solutions, prepared in 10 mM sodium phosphate buffer at different pH's*

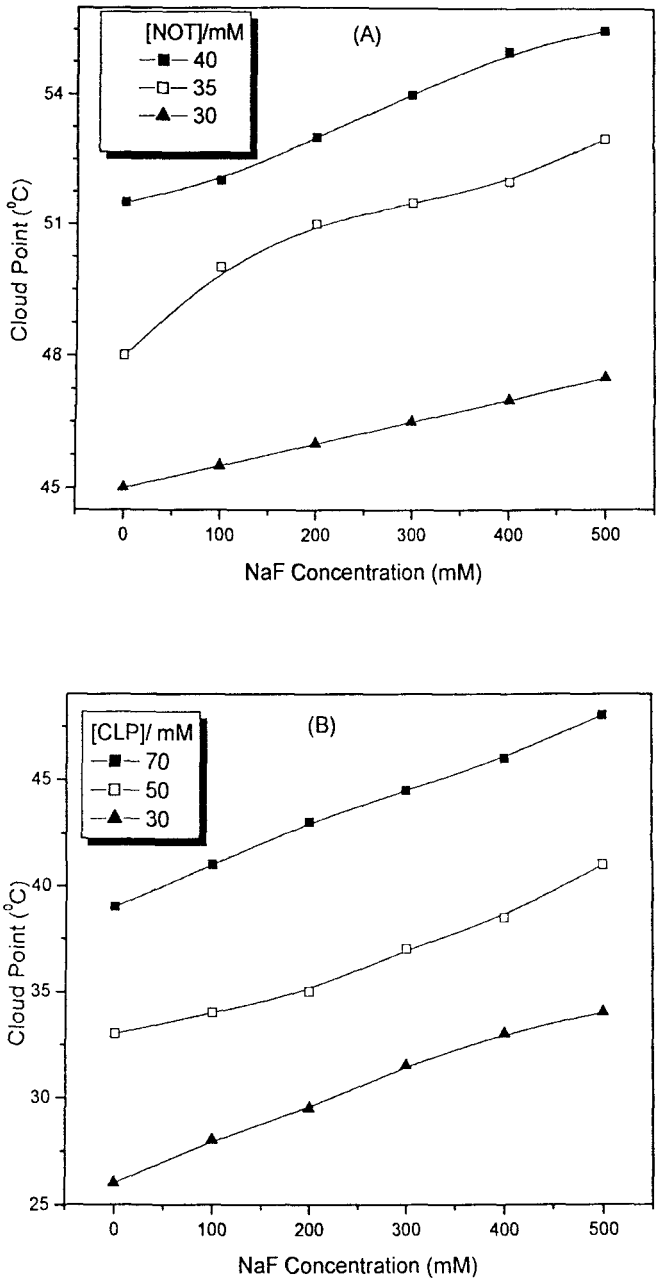

Fig. 4.47: *Effect of NaF concentration on the CP of solutions containing different fixed amounts of NOT (pH = 7.07) (A) and CLP (pH=6.25) (B), prepared in 10 mM sodium phosphate buffer.*

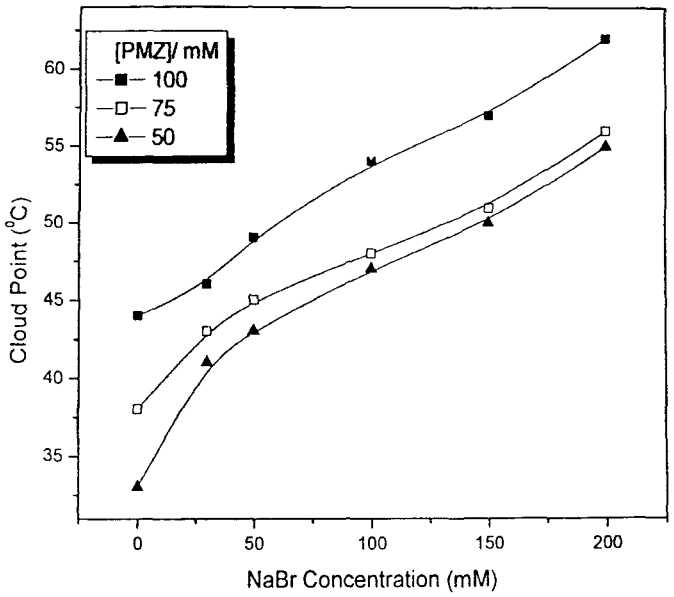

Fig. 4.48: *Effect of NaBr concentration on the CP of solutions containing different fixed amounts of PMZ (pH = 6.67), prepared in 10 mM sodium phosphate buffer.*

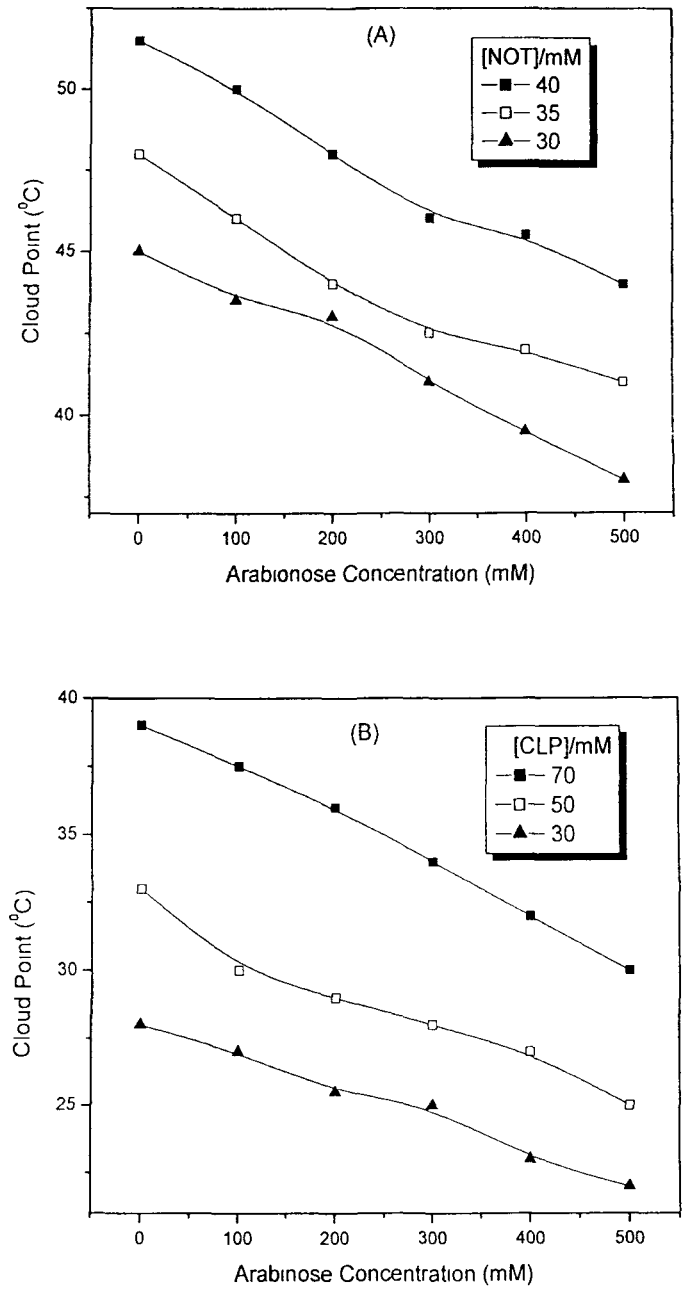

Fig. 4.49: *Effect of arabinose concentration on the CP of solutions containing different fixed amounts of NOT (pH = 7.07) (A) and CLP (pH=6.25) (B), prepared in 10 mM sodium phosphate buffer.*

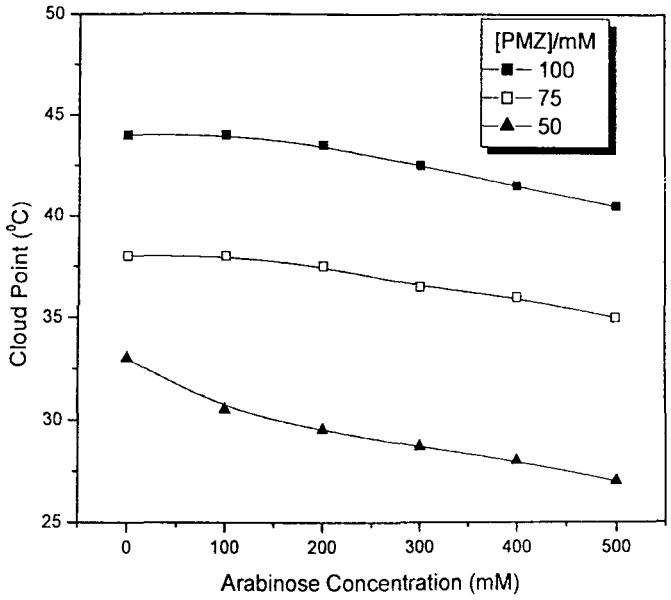

Fig. 4.50: *Effect of arabinose concentration on the CP of solutions containing different fixed amounts of PMZ (pH = 6.67), prepared in 10 mM sodium phosphate buffer.*

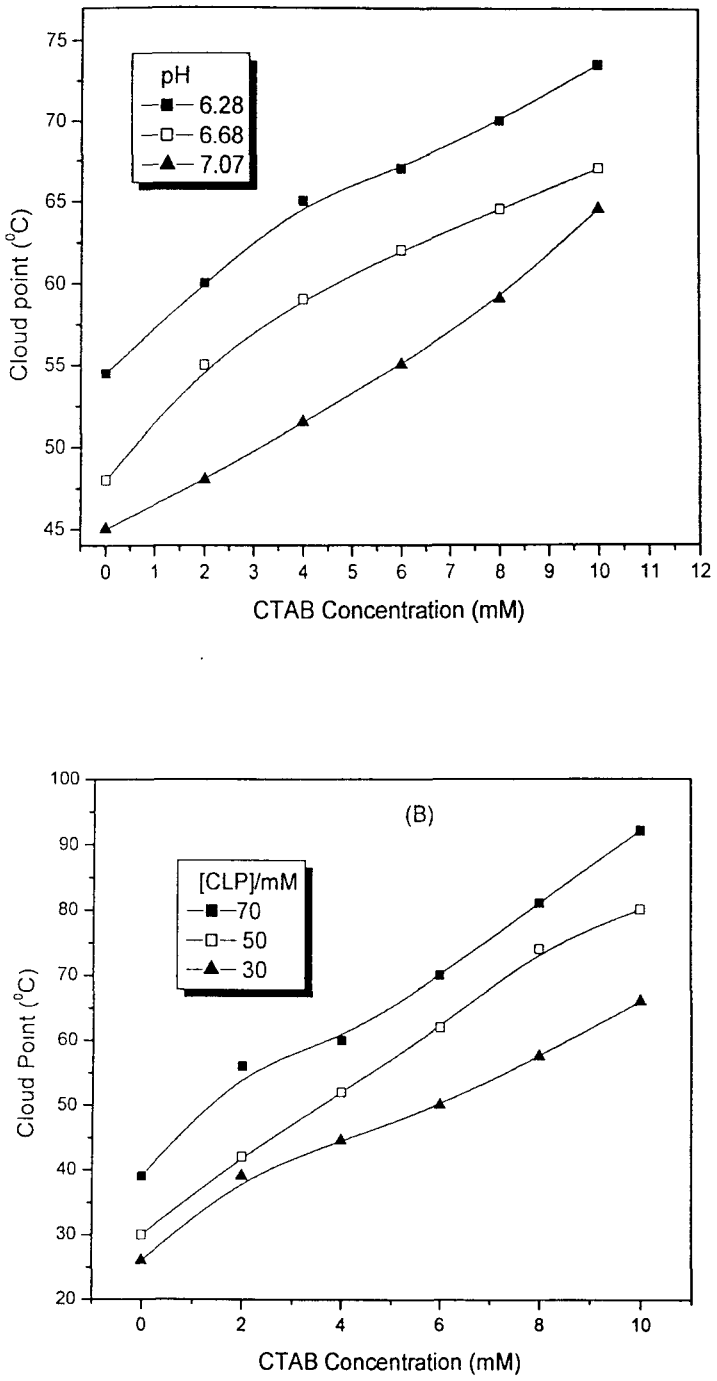

Fig. 4.51: *Effect of CTAB concentration on the CP of solutions containing different fixed amounts of NOT (pH = 7.07) (A) and CLP (pH=6.25) (B), prepared in 10 mM sodium phosphate buffer.*

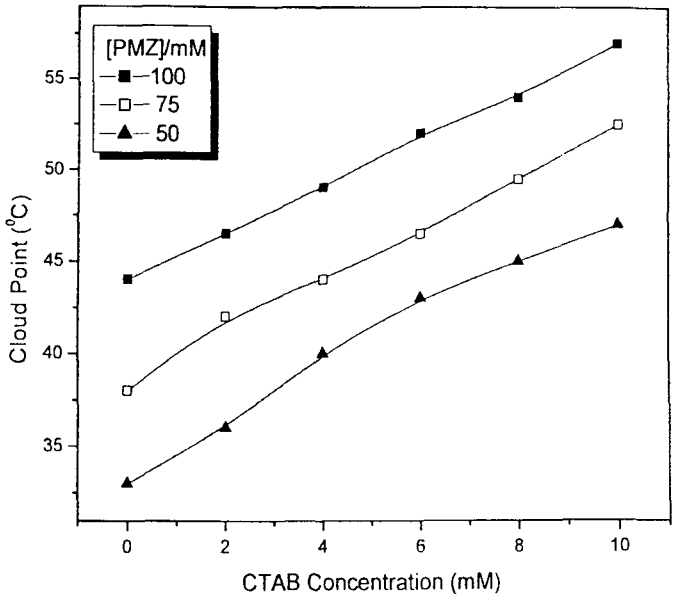

Fig. 4.52: *Effect of CTAB concentration on the CP of solutions containing different fixed amounts of PMZ (pH = 6.67), prepared in 10 mM sodium phosphate buffer.*

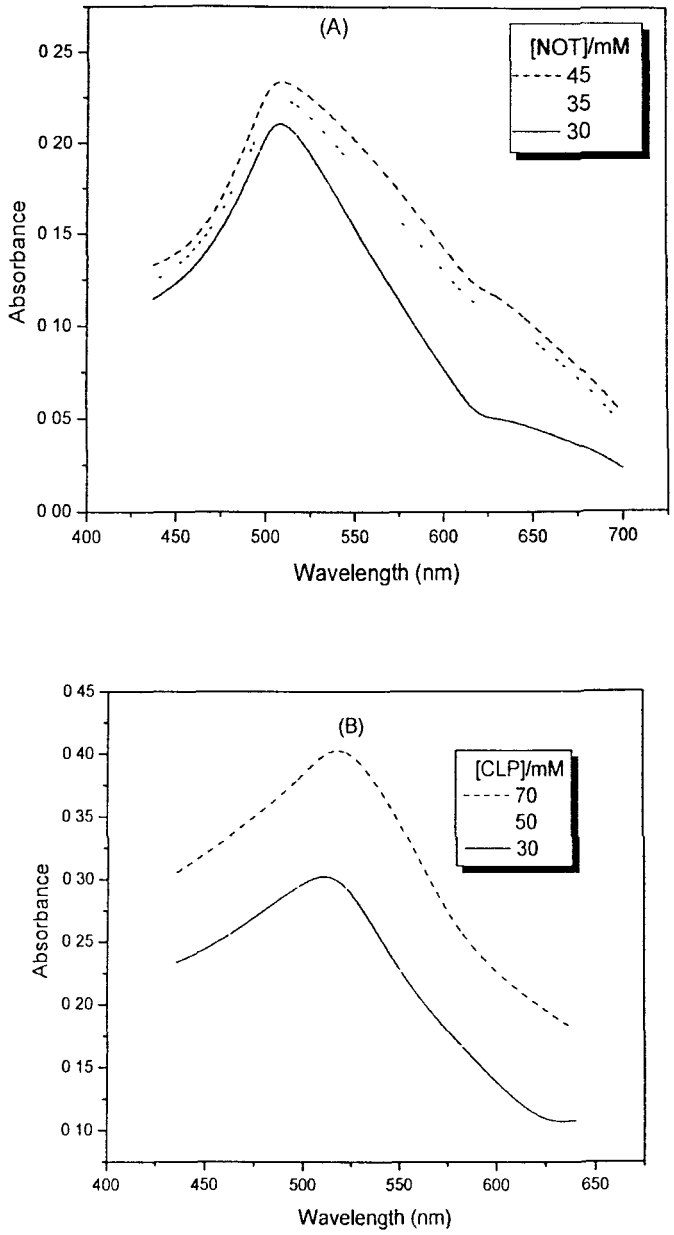

Fig. 4.53: *Visible spectra of Sudan III dissolved in drug solutions containing different fixed amounts of NOT (A) and CLP (B).*

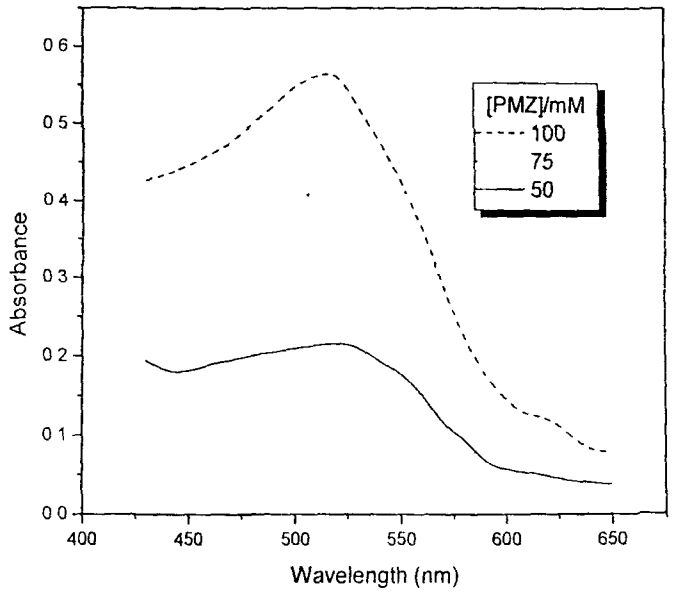

Fig. 4.54: *Visible spectra of Sudan III dissolved in drug solutions containing different fixed amounts of PMZ in water.*